A UNIQUE FACILITY TO TEST THE INFECTIVITY OF HUMAN-GENERATED AIRBORNE INFECTIONS

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ABSTRACT

Title: A unique facility to test the infectivity of human-generated airborne infections

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Tuberculosis (TB), one of the world's greatest killers, is predominantly spread by the airborne route. Drug-resistant *M. tuberculosis* has emerged as a global public health threat despite effective drugs and disease control strategies. Little is known about *M. tuberculosis* transmission and the efficacy of necessary environmental (engineering) interventions for infection control; particularly in light of the global HIV/Aids epidemic.

This thesis covers the development, validation and calibration of the unique Airborne Infection Research (AIR) facility (apparatus) that utilises a biological model to sample airborne *M. tuberculosis* by transporting infectious air from patient wards to animal exposure chambers housing guinea pigs. This capability, hitherto a universal limitation due to the unique characteristics of the tubercle bacili, will now allow a collaboration of researchers from around the world to undertake scientific studies to answer fundamental questions about the infectiousness of drug-resistant *M. tuberculosis* and the efficacy of various engineering interventions to minimise the spread of airborne disease. These experiments will provide the scientific blue-prints for design of safer health care facilities and the development of improved building and construction standards.

The AIR facility, recently completed as part of this study, was the culmination of a five year research project by a collaborative research team from the SA Medical Research Council, the Council for Scientific and Industrial Research, the Centers for Disease Control, Atlanta, USA; and Harvard University, Boston, USA; made possible with initial funding provided by the US Agency for International Development (USAID) and private sector donors which included the South African National Tuberculosis Association (SANTA).

The author, as the engineering research member of the collaborating research team, was responsible for all architectural engineering aspects of the research behind the design, development, operation and, in part, the bioaerosol sampling techniques; and had to develop an in depth appreciation and understanding of *M. tuberculosis* generation, risk and control in order to anticipate what was needed from the apparatus to support the various research projects that are to be undertaken.

The various engineering interventions necessary to curtail transmission of infection, such as ultraviolet germicidal irradiation (UVGI) and other electro/mechanical interventions can now be tested and evaluated. The facility, as an apparatus, is capable of supporting the experiments intended for the study of these interventions in that the effects of varying ventilation rates and environmental conditions, such as temperature and humidity on the transmission dynamics of aerosolized infectious particles, are possible.

The thesis discusses the hypothesis, aims, results and conclusions of the apparatus development, validation and calibration experiments of the unique state-of-the-art facility.

The effectiveness and airtightness (leakage factor) of the air distribution from the wards, the transporting capacity of gram-positive and negative aerosolized bacteria and the efficacy of the in-line UVGI units to the animal infection chambers were conclusively proven via validation experiments. The results presented indicate that from the validated operational parameters of the apparatus the losses were less than 5% for non-biological substances and less than 12% for endospores (*Serratia marcescens*). No significant losses were noted across the transfer axial fan. A 100% efficacy was achieved across the in-line ultraviolet germicidal irradiation units as no *Serratia marcescens* were detected in the animal room.

The calibration experiment, conducted to calibrate the exposure apparatus of the AIR facility in meeting its purpose to effectively transfer infectious airborne particles from patient wards (clinical unit) to the animal exposure chambers, concluded from the rate of guinea pig infections observed that the AIR facility is a highly effective way to quantify the infectiousness of TB patients. The high rate of observed infections among the guinea pig infections proves conclusively that the AIR facility will serve its purpose to effectively evaluate infectiousness of the ward air and to test the efficacy of engineering interventions to minimize the spread of the disease.

The AIR facility now provides unique opportunity to evaluate the efficacy of novel engineering interventions for infection control, particularly in light of the global HIV/Aids epidemic. Future studies that are planned are also discussed.

SAMEVATTING

Titel: ‘n Unieke fasiliteit om die oordraagbaarheid van menslike gegenereerde lugverspreide infeksies te toets

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Graad: Philosophiae Doctor in Engineering

Tuberkulose (TB), een van die wêreld se grootste oorsake van die dood, word hoofsaaklik deur die lug versprei. Veelvoudige Middel Weerstandige TB (VMW-TB) het na vore gekom as ‘n wêreldwye gesondheidsgevaar ten spyte van effektiewe medikasie en siektebeheer strategieë. Min is bekend oor *M. tuberculosis* oordrag en die effektiwiteit van die nodige omgewings- (ingenieurs-) ingryping vir siektebeheer, veral gesien in die lig van die wêreld-wye HIV/Vigs epidemie.

Die tesis handel oor die ontwikkeling, geldigheid en kalibrering van die unieke Lugverspreiding Infeksie Navorsing (AIR) fasiliteit (apparatuur) wat gebruik maak van ‘n biologiese model wat monsters neem van lugverspreide *M. tuberculosis* wat deur besmette lug versprei word van pasiëntsale na diere- blootstellingsvertrekke waar marmotte gehuisves word. Hierdie vermoë, tot dusver ‘n universiële beperking as gevolg van die unieke eienskappe van die tubercle bacili, sal nou samewerkende navorsers van regoor die wêreld die geleentheid gee om wetenskaplike studies te doen om die fundamentele vrae oor die oordraagbaarheid van *M. tuberculosis* te beantwoord en die effektiwiteit van verskillende ingenieursingrypings om die lugverspreiding van die siekte te beperk. Hierdie eksperimente sal die wetenskaplike bloudrukke vir die ontwerp van veilige gesondheidsorgfasiliteite voorsien sowel as die ontwikkeling van verbeterde bou- en konstuksiestandaarde.

Die AIR fasiliteit, wat onlangs voltooi is as deel van hierdie studie, was die hoogtepunt van ‘n vyfjaar navorsingsprojek van ‘n gesamentlike navorsingspan van die SA Mediese Navorsingsraad, die Wetenskaplike en Nywerheidsnavorsingsraad, Sentrums vir Siektebeheer, Atlanta, VSA; en Harvard Universiteit, Boston, VSA; wat moontlik gemaak is deur aanvanklike befondsing wat voorsien is deur die Amerikaanse Agentskap vir Internasionale

Ontwikkeling (USAID) sowel as privaatsektor donasies wat die Suid Afrikaanse Nasionale Tuberkulose Assosiasie (SANTA) insluit.

Die skrywer, as die ingenieursnavorsingslid van die gesamentlike navorsingspan, was verantwoordelik vir al die argitektoniese ingenieursaspekte van die navorsing agter die ontwerp, ontwikkeling, werking en gedeeltelik, die biolug monsternemingtegnieke, en moes 'n indiepte waardering en begrip van *M. tuberculosis* generering, risiko en beheer ontwikkel om te kon voorsien watter eise aan die apparatuur gestel sou word om die verskillende navorsingsprojekte wat onderneem word, te ondersteun.

Die verskillende ingenieursingrypings wat benodig is om die verspreiding van die siekte te beperk, soos ultraviolet ontsmettingsbestraling (UVOB) en ander elektro/meganiese ingrypings, kan nou getoets en evalueer word. Die fasiliteit, as apparatuur, kan die eksperimente wat bestem is vir die bestudering van hierdie ingrypings ondersteun in soverre die effek van veranderende ventilasiekoerse en omgewingstoestande, soos temperatuur en humiditeit op die oordragdinamika van lugverspreide infeksiedeeltjies, moontlik is.

Die tesis behandel die hipotese, doel, resultate en gevolgtrekking van die apparatuur ontwikkeling, geldigheid en kalibreringseksperimente van dié unieke vooraanstaande fasiliteit.

Die effektiwiteit en die lugdigtheid (lekkasie faktor) van die lugverspreiding deur die sale, die bewegingskapasiteit van gram-positiewe en negatiewe lugverspreide bakterieë, en die effektiwiteit van die in-lyn UVGI eenhede na die diere- blootstellingsvertrekke is onweerlegbaar deur die geldigheidseksperimente bewys. Die voorgelegde resultate bewys dat vanaf die gevalideerde operasionele parameters van die apparatuur die verliese minder as 5% van die nie-biologiese bestanddele en minder as 12% van endospore (*Serratia marcescens)* was. Geen noemenswaardige verliese is deur die oordrag inlynwaaier waargeneem nie. 'n 100% effektiwiteit is behaal oor die in-lyn ultraviolet ontsmettingsbestralingseenheid, aangesien geen *Serratia marcescens* in die dierevertrek gevind is nie.

Die kalibreringseksperiment, wat gedoen is om die tentoonstellingsapparatuur van die AIR fasiliteit te kalibreer om aan sy doel te beantwoord om lugoordraagbare infeksies effektief van pasiëntsale (kliniese eenhede) na die diere blootstellingsvertrekke oor te dra, het na aanleiding van die tempo van marmotinfeksies tot die gevolgtrekking gekom dat die AIR fasiliteit 'n hoogs effektiewe manier is om die besmetlikheid van TB-pasiënte te kwantifiseer. Die tempo

van die infeksies wat onder die marmot-infeksies waargeneem is, bewys uitsluitlik dat die AIR fasiliteit aan sy doel sal beantwoord om besmetlikheid van saallug effektief te evalueer en om die effektiwiteit van ingenieursingrypings te toets om die verspreiding van die siekte te verminder.

Die AIR fasiliteit voorsien nou 'n unieke geleentheid om die effektiwiteit van ongewone ingenieursingrypings van infeksiekontrole te evalueer, veral in die lig van die wêreldwye HIV/Vigs epidemie. Verdere beplande studies word ook bespreek.

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NOMECLATURE

C	Number of new cases	
S	The number of susceptibles	
I	The number of infectors	
r	The effective contact rate	
q	The quanta of airborne infection	
p	The volume of air breathed (by a susceptible)	[m^3]
V	Volume of an enclosed space	[m^3]
t	Time	[s]
τ_n	The system average nominal time constant (i.e. the ratio of the volume of the space to the rate of change of fresh air to the space)	[s]
τ_t	The turnover time (or residence time of the contaminant)	[s]
$M(\infty)$	The total mass of contaminant (at steady state)	[kg]
Q_s	The rate at which a volume of air is supplied to an enclosed space	[m^3/s]
Q_e	The rate at which a volume of air is vented from an enclosed space	[m^3/s]
Q_i	The rate at which a volume of air infiltrates an enclosed space	[m^3/s]
$\dot{q}$	Mass flow rate of quanta of airborne infection	[kg/s]
K	Rate of air removal in air changes per hour	[/s]
N_o	Initial concentration of airborne organisms	[/m^3]
N_t	Concentration at time t	[/m^3]
N_{eq}	The equilibrium concentration	[/m^3]
N_{ave}	The average concentration	[/m^3]
N_{ref}	The concentration at a reference point	[/m^3]

N_{duct}	The concentration in a duct	[/m^3]
N_{source}	The concentration from source	[/m^3]
ε_{CR}	Contaminant removal effectiveness	
ε_{v}	Ventilation effectiveness	
η_{v}	Ventilation efficiency	

ABBREVIATIONS

ACGIH: American Conference of Governmental Industrial Hygienists
ACH: Air Changes per Hour
AIHA: American Industrial Hygiene Association
AIDS: Auto Immune Deficiency Syndrome
AIR: Airborne Infection Research
ANSI: American National Standards Institute
ARV: Antiretroviral
ASHRAE: American Society for Heating, Refrigeration and Airconditioning Engineers
BMS: Building Management System
BRI: Building-Related Illness
BSL: Bio-safety Level
CADR: Clean Air Delivery Rate
CDC: Centers for Disease Control
CFD: Computational Flow Dynamics
CFU: Colony Forming Unit
CO_2: Carbon Dioxide
CSIR: Council for Scientific and Industrial Research
DB: Dry Bulb
DDC: Direct Digital Control
DNA: Deoxyribonucleic Acid
DOP: Dioctyl Phthalate Particles
DOT: Direct Observation Therapy
GAP: Global AIDS Program
HEPA: High Efficiency Particulate Arrestance
HHPC: Hand Held Particle Counter
HVAC: Heating Ventilation and Airconditioning
IES: International Environmental Sciences
IRPA: International Radiation Protection Agency
ISEE: International Society for Environmental Epidemiology

MCC: Motor Control Centre
MDR-TB: Multi-drug Resistant Tuberculosis
NHLS: National Health Laboratory Service
NBR: National Building Regulations
NBRI: National Building Research Institute
NIOSH: National Institute of Occupational Safety and Health
NIST: National Institute of Science and Technology
PPD: Purified Protein Derivative
OHS: Occupational, Health and Safety
PSL: Poly-Styrene Latex
REL: Recommended Exposure Limit
RH: Relative Humidity
RSE: Relative Spectral Effectiveness
SABS: South African Bureau of Standards
SA MRC: South African Medical Research Council
SANTA: South African National Tuberculosis Association
SARS: Severe Acute Respiratory Syndrome
SBS: Sick Building Syndrome
SET: Standard Effective Temperature
SPF: Special Pathogen Free
STG: Stage
TB: Tuberculosis
TLV: Threshold Limit Values
TOI: Toluene-di-isocyanate
TU: Tuberculin Unit
USAID: United States Agency for International Development
UV: Ultraviolet
UVGI: Ultraviolet Germicidal Irradiation
WB: Wet Bulb
WHO: World Health Organisation

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1

INTRODUCTION: THE PROBLEM AND ITS SETTING

Mycobacterium tuberculosis remains a significant airborne threat to workers worldwide, accounting for many millions of dollars of worker testing, prophylactic treatment, and infection control interventions annually. There is a need to be able to determine the infectiousness of MDR-TB patients and assess the efficacy of various clinical and engineering interventions on the transmission of M. tuberculosis. The limitation to testing such interventions is the inability to quantitatively culture human-generated airborne viruses and bacteria of interest from the air under real life conditions. The development of the unique International Experimental Tuberculosis Transmission Unit in South Africa, known as the Airborne Infection Research (AIR) facility, provides the essential apparatus necessary for the research on effective interventions to minimise the spread of the disease.

1 INTRODUCTION: THE PROBLEM AND ITS SETTING

1.1 Problem context

Tuberculosis (TB) is still among the world's greatest killer diseases.

South Africa faces one of the most devastating (TB) epidemics in the world, ranking 2nd in terms of TB incidence (or number of cases per capita) and 9th in terms of the overall TB burden (or total number of cases). A recent study by the South African Medical Research Council (SA MRC) revealed that 55% of TB patients in South Africa are also infected with the Human Immunodeficiency Virus (HIV), with the co-infection rate exceeding 70% in some provinces [1].

A serious complication of the TB problem in SA has been the emergence of Multi-drug Resistant Tuberculosis (MDR-TB) in all nine provinces since the mid-eighties. Estimates from drug resistance surveys conducted by the SA Medical Research Council (SA MRC) [1] in 2001-2002 indicate that the proportion of new patients with MDR-TB is now between 1% and 3%; among previously-treated patients this proportion is between 4% and 10% (Weyer, personal communication). These proportions are relatively low in comparison with rates found elsewhere; however; given the high burden of TB in the country, these proportions translate into more than 2 500 cases of MDR-TB diagnosed every year and there is reason to believe that the full brunt of MDR-TB has yet to be felt in SA [1].

Although TB is an old disease and has been fairly extensively studied, MDR-TB is a fairly new phenomenon and less is understood about the infectiousness, transmission dynamics and appropriate infection control procedures for this disease [2][3], particularly against the background of high HIV infection prevalence in most of the high-burden TB countries.

MDR-TB linked with HIV therefore has the potential to result in an uncontrollable epidemic with devastating economic and social consequences. Nosocomial spread of

MDR-TB has been documented in South Africa [4] [5] and there is growing concern among health care workers about their risk of contracting MDR-TB.

Whilst the increasing availability of antiretroviral (ARV) therapy in South Africa poses hope for the management of HIV-associated TB; ARV treatment rollout also brings together undiagnosed, often infectious TB (and MDR-TB) patients with immune-suppressed HIV-infected individuals, into hospitals, clinics and other congregate settings. Opportunities for transmission are therefore ideal. Transmission of TB and MDR-TB in communities and congregate settings has already been documented in several studies, invariably linked to HIV infection or affecting vulnerable groups such as children [1].

TB is an infectious disease transmitted from person to person by the airborne route, usually through coughing by a patient with active pulmonary TB. Infectious droplet nuclei containing tubercle bacilli may remain suspended in the air for prolonged periods of time, leading to a high risk of infection in congregate settings with poor or little ventilation where susceptible populations (e.g. children and immune-suppressed individuals) may be accommodated.

Newly revised and expanded guidelines from the Centers for Disease Control (CDC) in the USA to protect health care workers from occupational TB, attest to its ongoing importance in the workplace [6]. Globally, TB transmission; especially MDR-TB transmission; poses a serious threat in all institutional settings, from hospitals to prisons, especially among HIV-infected persons.

Other infections, such as influenza and Severe Acute Respiratory Syndrome (SARS), have been clearly shown to have airborne potential [7] [8], but the relative importance of the airborne route compared to large droplet spread remains unclear. Still other infections, such as anthrax and smallpox, while not normally significant airborne threats in nature, are potential biological weapons and could have devastating consequences in the workplace.

1.2 Delimiting the research problem

1.2.1 Preamble

Mycobacterium tuberculosis remains a significant airborne threat to workers worldwide, accounting for many millions of dollars of worker testing, prophylactic treatment and infection control interventions annually.

TB transmission is unpredictable and highly variable due to several factors: individual variability in infectiousness, environments more or less conducive to transmission, variable susceptibility of those exposed and highly variable exposure times. When, by chance, several factors favouring transmission occurred at the same time, widespread transmission was reported, whereas other apparently similar cases appear to infect no-one. The transmission factors from case to case are simply different.

1.2.2 Variable TB infectiousness as a barrier to clinical research

Were one to attempt a simple hospital study to compare infection rates among staff on floors with and without air disinfection, any differences observed may be due to the protective effects of air disinfection, but in fact would more likely reflect the chance appearance of one or more highly infectious cases on the floor, or prolonged exposure to unsuspected cases of ordinary infectiousness.

Very large, multi-centre trials would be needed for such occurrences to happen equally, by chance, in both control and experimental settings. Currently, no gold standard exists to certify that MDR-TB patients on treatment are no longer infectious.

The relative infectiousness of MDR-TB therefore involves much scientific debate and controversy. The genetic mutations leading to MDR-TB can in theory reduce the fitness and transmissibility of MDR-TB strains; however; MDR-TB patients usually respond much slower to treatment and remain infectious for longer periods of time than drug-susceptible TB patients. Explosive outbreaks of MDR-TB in HIV-infected individuals have been recorded, suggesting that, even with reduced fitness and

transmissibility, drug resistant *M. tuberculosis* strains may be highly infectious or virulent in immune compromised individuals.

Laboratory tests such as sputum smear microscopy that demonstrate decreased numbers of organisms are used to estimate the point at which patients are consider not to be infectious any more; however; no data exists to correlate this with actual non-infectiousness. Proof of the duration of infectiousness of MDR-TB patients would have a major impact in both developing and industrialized countries, reducing the risk of drug resistant *M. tuberculosis* transmission and avoiding prolonged hospitalization of patients.

Comprehensive infection control packages are expensive and may not be affordable in developing country settings. How long MDR-TB patients should remain isolated to prevent transmission is not known, but is essential in resource-limited settings. There is a need to be able to determine the infectiousness of MDR-TB patients and assess the efficacy of various clinical and engineering interventions on the transmission of *M. tuberculosis*. This will allow for rational design of affordable infection control policies and procedures in all settings.

1.2.3 The need to assess the efficacy of available engineering based interventions

Guidelines for prevention of nosocomial transmission of *M. tuberculosis* exist [9] [10]; however; evidence for the effectiveness of various interventions is lacking [11] [12].

Well documented episodes of TB transmission within hospitals and other institutions, many with MDR-TB, have led to well-founded concerns about transmission and recommendations and regulations to prevent nosocomial spread. The currently recommended engineering interventions in particular are expensive, often difficult to implement and potentially disruptive of patient care. The problem is compounded by the absence of scientific evidence that any of the recommended interventions are effective in practice.

There have been no epidemiological field trials of the engineering strategies, such as enhanced ventilation, directional airflow (negative pressure isolation), high efficiency particulate arrestance (HEPA) air filtration or ultra-violet (UV) air disinfection in rooms or ventilation ducts. Proof that personal respirators of any kind will significantly prevent TB transmission is also lacking.

The absence of proof of efficacy reflects not only the neglect of TB research in recent decades, but also the erratic nature of tuberculosis transmission that defies study by ordinary clinical investigations.

The limitation to testing such interventions is the inability to quantitatively culture human-generated airborne viruses and bacteria of interest from the air under real life conditions.

1.2.4 The problem of sampling and culturing *M. tuberculosis* from the air

Tubercle bacilli cannot be cultured from the air because of their low concentration and slow growth rate relative to other ambient, more rapidly growing micro-organisms [18].

The slow growth of tubercle bacilli and low concentrations in air require long sampling periods during which culture media, even with selective antibiotics to suppress microbial growth, become overgrown with fungi and other bacteria. Whilst molecular amplification methods can detect nucleic acid from tubercle bacilli in the air, they cannot distinguish living from dead organisms nor quantify those with infectious potential [18].

It is therefore not possible to measure infectiousness of *M. tuberculosis* or drug resistant *M. tuberculosis* directly, nor can the efficacy of environmental infection control interventions to reduce or prevent transmission be measured directly.

Micro-organisms grown in culture have been artificially aerosolized in various media at high concentrations in small or large chambers and successfully collected with

mechanical air sampling over relatively short periods [13]. Recent studies have used this approach to test upper room various air disinfection technologies, but is unclear whether the results accurately predict the protection possible against aerosol generated by humans episodically coughing and sneezing from respiratory infections under real life conditions [14] [15].

Surrogate test organisms such as *Escherichia coli* and *Serratia marcescens* have long been used in aerobiology when the organism of interest is unavailable, hazardous or difficult to grow [16] [17] [20] [21]. As a prototype airborne infectious pathogen, *M. tuberculosis* can be used to study interventions aimed at a variety of other agents that are completely or partially airborne.

Unlike influenza and other respiratory infections that are often transient and seasonal, human-generated TB aerosols can be studied at any time due to the chronic nature of the disease and its unfortunate high prevalence in many parts of the world, in particular South Africa.

1.2.5 The hypotheses for the unique animal model

The need to undertake studies of drug resistant *M. tuberculosis* transmission under controlled conditions, enabling the assessment of the potential of MDR-TB bacilli for airborne dispersal, airborne survival and ability to generate infection and disease in guinea pigs exposed to air containing these organisms has long been identified.

The exquisite susceptibility of guinea pigs to TB infection and rapid progression to disseminated active disease; however; mimic the pathogenesis of TB in HIV-infected patients, thereby creating an ideal biological model for studying TB infectiousness and transmission dynamics under controlled conditions.

Quantitative experiments have shown that guinea pigs are highly susceptible to TB infection and disease when air containing infectious droplet nuclei is inhaled, with the probability of infection proportional to the concentration of infectious droplet nuclei in the air and the volume of air breathed over the duration of exposure.

Using the well-established method of guinea pig air sampling described above, human generated TB airborne transmission can be studied quantitatively and the effectiveness of various interventions measured during time controlled exposure experiments. As guinea pigs are highly susceptible to as little as a single inhaled droplet nucleus of human *M. tuberculosis*, they are unaffected by background airborne contaminants that make culture impossible [19]. Knowing their average minute ventilation, guinea pigs can therefore serve as quantitative air samplers for airborne infectious droplet nuclei generated by patients [20].

TB infection in the guinea pig can be easily determined by conducting tuberculin skin tests using purified protein derivate (PPD) after a few weeks of exposure. Progression to active TB disease occurs rapidly in infected guinea pigs, making these experimental animals very useful as proxy indicators of *M. tuberculosis* transmission.

Interventions that successfully protect guinea pigs from infection should be equally effective in protecting humans (including immune-suppressed individuals) as well, providing the much-needed scientific evidence for the efficacy and impact of environmental infection control measures. Of particular relevance for Africa and other resource-limited, high TB-HIV burden settings, is the development of evidence-based strategies for appropriate and affordable infection control in congregate settings such as hospitals and prisons.

1.3 Motivation for the study: an international experimental tuberculosis transmission unit in South Africa

The motivation for an experimental transmission unit in South Africa was to study the transmission dynamics of drug resistant *M. tuberculosis* and the associated public health benefits of appropriate infection control interventions in congregate settings and vulnerable populations.

The unfortunate high prevalence of MDR-TB in South Africa presents the opportunity to definitively study *M. tuberculosis* transmission and engineering interventions.

Because of the above-mentioned erratic nature of TB transmission, well designed clinical trials require a large number of exposures confined to the spaces protected by such interventions with appropriate controls. Due to the decrease of reported cases in the USA (26% decrease in the five years, between 1992 to1997), intervention experiments were greatly hampered.

For this reason the advances in the control of TB and other airborne infections have been somewhat stagnant, hampered greatly by the inability to quantitatively culture organisms from room air. There have been no successful attempts to quantitatively recover Tubercle Bacilli from room air under clinical conditions since the Wells and Riley's classic experiments employing guinea pig air sampling almost 40 years ago.

The classic tuberculosis experiment, using guinea pigs as a biological model, was envisioned by William Firth Wells. This study was first developed and operated by Richard L. Riley and colleagues [21] [22] [38] [39] [40] [41] in the late 1950s and early 1960s in Baltimore. Riley's pioneering work proved for the first time that TB is airborne, correlating transmission to clinical and bacteriologic factors, including cough frequency, lung cavitation, drug resistance and response to treatment.

He also showed that germicidal irradiation in ductwork completely prevented infection of the guinea pigs in one of the two colonies. This work provided the inspiration for the development of a unique facility in South Africa to test the infectivity of human generated airborne infections.

Although Riley's technique is a proven technology, its requirement of a specialized hospital ward with at least several infectious patients with all exhaust air delivered to large numbers of guinea pigs in exposure chambers, has since been difficult to replicate in the USA. However; whilst TB rates are falling in the USA, the disease is spiralling out of control in developing countries where resources for treatment are limited.

The development of the unique International Experimental Tuberculosis Transmission Unit in South Africa, known as the Airborne Infection Research (AIR) facility,

covered in the study, provides the essential apparatus necessary for the research on effective interventions to minimise the spread of the disease.

The advent of advanced technologies for environmental (engineering) control equipment and direct digital (electronic) controls, provide for a unique more advanced apparatus than that used by Riley, permitting greater insights and thus answers to questions on transmission and interventions from the various experiments that have been planned, than were possible decades ago on Riley's ward.

The AIR facility now serves as the primary apparatus required for the research projects covering various studies into TB undertaken by the MRC, the Council for Scientific and Industrial Research (CSIR) (North West University), CDC and Harvard University in collaboration.

The AIR facility, operated as an apparatus to study transmission, extracts air from MDR-TB patient wards and transfers this pathogen contaminated air to exposure chambers housing guinea pigs which serve as quantitative samplers of the human-generated infectious aerosols. Measuring the number of guinea pigs infected over time, linking guinea pig infections to individual patients by means of molecular techniques provides for the unique sampling strategy hypothesised above.

Actual patients provide the source of airborne *M. tuberculosis* droplet nuclei, the vehicles of transmission. These patients occupy the AIR facility wards for variable periods of time, temporarily serving as sources of airborne organisms as therapy is begun. Patients on therapy who became non-infectious are then replaced by newly admitted, infectious cases.

The design of this unique facility facilitates scientific studies to answer fundamental questions about the infectiousness of drug resistant *M. tuberculosis*, the role of HIV and the effectiveness of environmental controls to curtail transmission. The apparatus has been designed to support the many experiments on the effects of varying ventilation rates and environmental conditions such as temperature and humidity on transmission dynamics of aerosolized infectious particles.

The efficacy of engineering interventions against the transmission of infection, such as ventilation (Dilution techniques), ultraviolet germicidal irradiation (UVGI) (Disinfection techniques) and other electro/mechanical interventions will be evaluated.

1.4 Review of relevant literature

1.4.1 Introduction

A review of relevant literature guiding current decisions on the biological procedures to be employed in the first experiment in the AIR facility is provided. The review also sets the necessary background for the study.

The literature related to the study falls into the following main categories:

1.4.2 Statutory and regulatory requirements for health and safety in the built environment

In South Africa, if work is performed where the potential for occupational exposure to bio-hazardous materials exists, bio-safety hazard safety shall be considered. The Occupational, Health and Safety (OHS) Act (Act 85 of 1993) which includes Regulation *No R1390:* 27th December 2001, applies. *(These Regulations apply to Hazardous Biological Agents (HBA).)*

Hazardous Biological Agents are defined as "infectious" agents, or materials produced by living organisms that may cause disease in other living organisms.

The National Building Regulations (NBR) [24], apply to all buildings in South Africa. The NBR is supplemented by the South African Bureau of Standards (SABS) 0400-1987 [25] (as amended), which provides the minimum ventilation requirements, such as outdoor air quantities and the flow of air for occupied spaces, which would be "deemed to satisfy" the clauses of the NBR.

These specified parameters were established to ensure acceptable levels of carbon dioxide concentrations in the occupied spaces whilst controlling odour and providing occupant comfort; the premise being that with carbon dioxide control, general contaminant control is also achieved.

When applied to the design of heating, ventilating and airconditioning (HVAC) systems for buildings, good engineering practice will ensure acceptable air quality and thermal comfort at reasonable capital and operating costs. Acceptable air quality implies adequate provision of oxygen, removal of respired carbon dioxide and other contaminants. Contaminants could include non-specific odours, vapours, gases or aerosols.

When reviewing the South African National Building Research Institute (NBRI) design specifications/briefs; however; (which are in line with the above NBR) the provision of natural, rather than mechanical ventilation for most buildings, has been promoted as adequate.

Whilst it is believed that in most geographical areas in South Africa a satisfactory indoor thermal environment can be achieved via the "good design" of the structure and natural ventilation via opening windows alone, it is accepted that HVAC is required in certain healthcare facility areas, such as operating theatres, intensive care units and burn units.

The above applies to most building types including general hospital areas, such as in-patient facilities and general congregate spaces; the premise being based on anticipated climatic conditions alone.

In addition, the Standard Effective Temperature index (SET), devised by the American Society of Heating, Refrigerating and Airconditioning Engineers, Inc. (ASHRAE) [26] and recommended by the NBRI for use in evaluating indoor thermal conditions when developing conventional forced ventilation systems, is most inappropriate when considering natural ventilation as an intervention against airborne infectious diseases.

1.4.3 Indoor air quality: International norms and guidelines

ASHRAE has defined acceptable indoor air quality as air in which there are no known contaminants at harmful concentrations as determined by cognizant authorities and with which a substantial majority (80% or more) of the people exposed, do not express dissatisfaction [27].

Whilst these accepted norms and guidelines, under ideal conditions, hold true for the comfort of occupants in conventional commercial and institutional buildings, they fall far short when special factors that mitigate indoor air quality problems are present. These problems often result in occupant threat, as in the case with healthcare providers in facilities for treating airborne infectious diseases.

Historical ventilation guidelines were intended, in part, to reduce the risk of airborne infections (caused by *M. tuberculosis* in particular), but were only estimates. While there are many examples of poor building ventilation associated with high rates of transmission of airborne infections, such as influenza and TB, no scientific basis for ventilation standards to reduce the transmission of such airborne pathogens has been developed to date.

It is reasonable to assume therefore that more ventilation should progressively reduce the risk, but there does not appear to be a threshold for complete safety. Moreover, there are published examples of infections, such as measles, where adequately functioning ventilation systems have been conduits for extensive spread of contaminants and biological agents throughout buildings.

1.4.4 Effective TB infection control programmes as recommended by the Centers for Disease Control (CDC)

In its *Guidelines for Preventing the Transmission of Mycobacterium Tuberculosis in Health-Care Facilities*, published in 1994, the US Centers for Disease Control (CDC) [28] indicate that a tuberculosis infection control programme should be based on a hierarchy of control measures.

These include:

1. Administrative measures such as work practices, policies and procedures, education and training, TB screening of healthcare workers and appropriate utilisation of existing facilities.
2. The implementation of engineering controls.
3. Personal respiratory protection in specified areas where there is a high risk of TB exposure.

The CDC [28] guidelines, when discussing the implementation of engineering controls, stipulate that an effective tuberculosis-infection control programme includes the design of ventilation systems, which serve isolation rooms to achieve six air changes per hour (ACH) for existing facilities and approximately twelve ACH in new or renovated facilities.

The air changes per hour (ACH) are defined as the number of times air is theoretically replaced in a defined space during one hour.

The minimum of six ACH was chosen because three recognised authorities, including ASHRAE [27], recommend it as the ventilation rate for tuberculosis isolation rooms. The CDC confirms that this is a recommendation based on comfort and odour control considerations rather than health and safety considerations.

In these guidelines the CDC do; however; state that the effectiveness of these levels of ventilation in reducing the concentration of droplet nuclei in the room has not been directly evaluated. Further, whilst it is clear that ventilation rates greater than six ACH will provide a greater reduction of droplet nuclei concentration than lower ACH, an accurate quantification of the decrease in risk with increasing rates of ventilation has not been performed and may not be possible.

A major disadvantage of dilution ventilation is that large volumes of dilution air must be supplied to the space. It can be costly to move and condition (heat or cool) these

large volumes of air. Perhaps, even more importantly, it is extremely difficult to control worker exposure near the source of the contaminant where dilution has not yet occurred.

Whilst international guidelines such as those on minimizing TB transmission are available, little guidance is provided for the planners, designers and managers of healthcare facilities in resource-limited settings. (This is particularly evident when reviewing the South African National Tuberculosis Control Programme Practical Guidelines).

Infection control in high HIV-prevalence settings is of paramount concern; however; because of resource-constraints in such settings, proven interventions need to focus on relatively inexpensive administrative and basic environmental measures. Where such needs have been identified and the use of certain commercially available interventions proposed, it is not generally appreciated that the scientific evidence for the effectiveness of certain of these interventions is lacking.

1.4.5 Patient infectiousness

Clinical characteristics likely to differentiate highly infectious patients include sputum smear positivity, presence of cavitary disease on chest radiography, the strength and frequency of cough and the characteristics of sputum specimens produced. The relative infectiousness of (drug-susceptible) HIV-positive- and -negative TB patients with positive acid-fast microscopy smears have been confirmed in several epidemiologic studies [29] [30]. The correlation between the number of bacilli found in sputum specimens and the potential for infectiousness has also been documented, rendering sputum microscopy useful as a robust test to rapidly identify highly infectious patients.

Patients with cavitary disease harbour large numbers of bacilli often resulting in positive smears [31]. Smear conversion is sometimes delayed in patients with cavitary disease, suggesting possible prolonged infectiousness, although cultures may turn negative earlier than smears [31]. Limited evidence from MDR-TB patients

indicates prolonged culture positivity in patients with cavitary disease, suggesting a higher potential for transmission [31].

Cough frequency has been suggested to be an indicator of infectious aerosol production, with work by Fennelly *et al* [32] suggesting a trend correlating cough frequency with culturable aerosols. Earlier epidemiological studies; however; suggested cough frequency to be less of an index of infectivity than the bacteriologic status of the patient [33].

1.4.6 Guinea pig infection characteristics

Observed experimental infections in animal models are dependent on the variables of the particular testing systems being employed, rendering extrapolation from previous studies difficult. Nevertheless, available evidence shows that low-dose experimental aerosol challenge of guinea pigs with virulent *M. tuberculosis* is characterised by an initial lag phase with lack of multiplication for 5 to 7 days [34] [35]. Multiplication occurs within the following 10 to 14 days resulting in up to 75% of guinea pig lung lobes being infected. Subsequent lymphatic dissemination results in isolation of viable bacilli in the bronchial lymph nodes approximately 10 to 14 days post-challenge. Seeding of bacilli from the lymphatics into the bloodstream, results in recovery of bacilli from the spleen and other tissues 14 to 21 days post-challenge.

The first detectable viable *M. tuberculosis* can be recovered from lung lobes displaying primary lesions, these being present from day 16 post-challenge. In addition, isolates can be obtained from primary lesion-free lung lobes from day 22 post-challenge, as a result of miliary spread. Presence of primary lesions are indicative of a high recovery rate of bacilli during the first 30 days post-challenge; however; bacilli can also be recovered from primary lesion-free lobes after 41 days post-challenge [36].

The appearance of large numbers of bacilli in the lymph nodes, spleen and all lobes of the lungs has been associated with the onset of hypersensitivity to mycobacterial antigens [34]. This is consistent with the finding that guinea pigs appear unresponsive

to purified protein derivative (PPD) for the first two weeks post-challenge and only begin to respond immunologically by week three (days 17 - 26), as measured with PPD RT23, 1 tuberculin unit (TU) and induration diameter 10 - 13 mm [34] [35]. However; no consensus has been established on the type of tuberculin, dose, or administration schedule required to indicate an optimum response to infection.

Guinea pigs to be used in the AIR facility are special-pathogen-free (SPF) and will receive sterilised food and water, resulting in virtually no risk of non-specific hypersensitivity reactions. Therefore, use of 100TU with reading at 24 hours, using any reaction as indication of infection, will be the departure point for the first experiment, to be adjusted if dictated by the data obtained.

1.5 Specific aim of the study

1.5.1 Preamble

Faced with the threat of MDR-TB there is a need not only to test conventional approaches to protecting workers from airborne infections, but to develop more effective technologies and to test them in a standardized way as they become available.

The inability to quantitatively culture human-generated *M. tuberculosis* from the air under real life conditions as discussed in the foregoing paragraphs, is the limiting factor to scientifically test the efficacy of such engineering interventions in minimizing transmission of the airborne contagion.

The aim of this study is the development, the validation and calibration experiments of the unique International Experimental Tuberculosis Transmission Unit in South Africa, known as the Airborne Infection Research (AIR) Facility (AIR facility).

The operation of the facility involves the extraction of infectious air from 6 MDR-TB patients accommodated in the in-patient hospital wards and transferring this air to

exposure chambers housing guinea pigs, which serve as living quantitative samplers of the human generated *M. tuberculosis*.

1.5.2 The development of an apparatus for measuring MDR-TB transmission and infectiousness

Hypothesis: *By developing a facility, using the well-established method of guinea pig air sampling, human generated TB airborne transmission can be studied quantitatively and the effectiveness of various interventions measured during time controlled exposure experiments.*

Specific aim: *To develop an Airborne Infection Research (AIR) facility to study the transmission dynamics of MDR-TB and associated public health benefits of appropriate infection control interventions in congregate settings and vulnerable populations using a biological model.*

As discussed above the *M. tuberculosis* bacilli cannot be cultured from the air because of their low concentration and slow growth rate relative to other ambient, more rapidly growing micro-organisms. Molecular amplification methods can detect nucleic acid from tubercle bacilli in the air, but cannot distinguish living from dead organisms nor quantify those with infectious potential.

The specific aim of the study was to develop an Airborne Infection Research (AIR) facility to study the transmission dynamics of drug resistance *M. tuberculosis* and associated public health benefits of appropriate infection control interventions in congregate settings and vulnerable populations using a biological model. This goal was pursued through specific research objectives achievable through the unique features of the facility.

The AIR apparatus is designed to undertake quantitative experiments by exposing guinea pigs to TB infection and disease via air containing infectious droplet nuclei. The probability of the guinea pigs acquiring infection being proportional to the effective contact rate, which is the function of the quanta of infectious droplet nuclei in the air and the volume of air breathed over the duration of exposure.

As the animals cannot be housed in the wards alongside patients, an integral animal unit, housing two animal exposure chambers with capacity to house 180 guinea pigs each, is completely separated from the clinical unit. Air is exhausted from the patient wards and common area via the "infected exhaust air transfer duct system", to the animal exposure chambers under preset controlled parameters to model conditions within the patient areas (these parameters are automated and may be modified to suit each experiment).

Conclusion on how to operate the mechanical apparatus to ensure that the guinea pigs are exposed to the highest quanta of aerosolised *M. tuberculosis* in the exposure chambers is based on and derived from the understanding of the "epidemiology of airborne infection" and "principles of control of airborne infection" [37].

By combining microbiology with engineering, a better understanding of the spread of infectious airborne diseases can be achieved. The engineering sophistication of the facility allows therefore for the development of scientific blueprints for TB treatment and in particular the design of safer healthcare facilities when considering the spread of infectious airborne diseases.

1.5.3 The validation of the unique Airborne Infection Research (AIR) facility

Hypothesis: *If the air tightness and efficiency (leakage factor) of the air transfer apparatus and the efficacy of the in-duct Ultra-Violet Germicidal Irradiation units were appropriately validated, the rates of infection in guinea pigs would reflect the concentration of infectious particles during the calibration experiment.*

Specific Aim: *To validate the exposure apparatus of the AIR facility in its purpose to effectively transfer infectious airborne particles from patient wards to the animal rooms, resulting in infection of adequate numbers of guinea pigs.*

In order to be certain that infection rates in guinea pigs accurately reflect the concentration of infectious particles in the exhaust air from the AIR clinical unit, it is necessary to validate the tightness and efficiency (leakage factor) of the transport apparatus. The efficacy of the induct UVGI units to the animal infection chambers, a

feature that allows one or both chambers to be protected from contagion on the ward should that be necessary for any particular experiment, will also have to be validated.

1.5.4 The calibration of the unique Airborne Infection Research (AIR) facility

Hypothesis: *That the transmission dynamics of the ward air is measurable using the biological model based AIR facility.*

Specific Aim: *To calibrate the exposure apparatus of the AIR facility in its purpose to effectively transfer infectious airborne particles from patient wards to the animal rooms, resulting in infection of adequate numbers of guinea pigs.*

Research in the newly-established Airborne Infection Research (AIR) facility in Witbank, South Africa will continue where the seminal Wells/Riley [38] [39] [40] [41] experiments on transmission dynamics of *M. tuberculosis* left off, focusing on currently unknown aspects of drug resistant *M. tuberculosis* transmission, within the context of HIV as a significant contributor to the epidemiology of TB in South Africa.

Based on the above historic Baltimore Veteran's Administration Hospital experiments on airborne transmission [38] [39] [40] [41], the unique state-of-the-art research facility (apparatus), which efficiently delivers infectious aerosols generated by TB patients on a six-bed experimental ward to two large exposure chambers containing a total of up to 360 pathogen-free guinea pigs, was designed and developed to address some of the current unanswered questions around the spread of tuberculosis.

The AIR facility enables the study of drug resistant *M. tuberculosis* transmission under controlled conditions, enabling assessment of the potential of MDR-TB bacilli for airborne dispersal, airborne survival and ability to generate infection and disease in guinea pigs exposed to air containing these organisms. Clinical predictors of patient infectiousness (e.g. active coughing, sputum smear-positive disease, cavitary disease on chest x-ray) can be assessed in addition to the overall duration of infectiousness.

The first experiment conducted at the AIR facility was aimed at biological calibration of the facility (e.g. ensuring that adequate numbers of guinea pigs are infected) and at

standardisation of biological procedures for patient and guinea pig investigations. This experiment will involve the collection of air from wards housing patients with confirmed MDR-TB and transporting such via a dedicated mechanical ventilation system to guinea pig exposure chambers. The ventilation rate from the wards into the exposure chambers will be at a preset rate of air changes per hour to achieve the maximum transfer of aerosols.

In this first experiment, the evaluation of the infectiousness of MDR-TB patients will be conducted and is designed to ensure maximum aerosolisation potential of the infection chamber supported by comprehensive patient and guinea pig investigations.

Results from the first experiment will be used to guide protocol development for subsequent experiments, as currently available published information relates primarily to drug resistant *M. tuberculosis* in the absence of HIV infection in patients and using less sophisticated systems for measuring airborne transmission of infection. Biological variables expected to be influenced in subsequent experiments, include the number of guinea pigs infected (which would influence sample size calculations), intervals of patient specimen collection and the relevance of sputum microscopy and culture as predictors of infectiousness. Required changes to the engineering components of the AIR facility exposure chamber will also be informed by results from the initial experiment.

The biological calibration of the AIR facility was therefore necessary to demonstrate that infectious particles can be successfully transferred from the clinical unit to the animal exposure chamber and cause infections in the guinea pigs and at standardisation of biological procedures for patient and guinea pig investigations.

1.6 Contributions of the study

From the extensive literature survey undertaken, it was confirmed that no apparatus exists universally to measure the infectivity of air and to test the efficacy of engineering interventions to minimize the spread of disease [18].

The primary contribution of this study is *the unique apparatus which will provide those in the scientific community who are concerned with the spread of infectious airborne disease (microbiologists, epidemiologists, engineers and healthcare providers), the capability of sampling M. tuberculosis from the air (hitherto a universal limitation due to the unique characteristics of the M. tuberculosis bacili* [18]*)*.

By linking the critical commonalities between the Biological Sciences with the fundamentals of the Engineering Sciences, such as Aerosol technology, Fluid Dynamics and Thermodynamics etc., the author was able to develop the essential elements for the apparatus to test human-generated airborne infection. The contributions to infection control via such a multi-disciplinary research process have received worldwide attention.

This unique contribution to bioaerosol science *has since been recognised by the Department of Health and Human Services, the Centers for Disease Control and Prevention (CDC) and the National Institute for Occupational Safety and Health (NIOSH), in the USA*, by awarding Dr E Nardell from Harvard University (one of the members of the collaborative research team), a $1 300 000 contract to undertake various ground breaking research projects at the facility over a period of five years, commencing in 2007. The project title will be: *The Testing Interventions to Human-Generated Occupational Airborne Infections.* Dr E Nardell [1] [42] [43] [44] will be the principal investigator, leading the collaborating team of international researchers noted above. The author will be the researcher responsible for all engineering aspects of the research.

By adopting the above-mentioned multi-disciplinary approach to this study (Engineering and Bio-Sciences), the author has proceeded with a study *to challenge the hypotheses of the Wells and Riley [50] equation on the probable rate of infection (Appendix D refers).* In order to reach an understanding of the effective contact rate with respect to the transmission of *M. tuberculosis,* the equation, which Wells and Riley created, has been theoretically developed in Chapter 2 of this thesis, using the

steady state conditions assumed by Wells and Riley. These assumptions were necessary as no method or apparatus to measure the necessary variables have existed.

From the conclusions of the investigation of the Wells/Riley equation to determine the spread of airborne infection (Appendix D), using the sample data observed during the calibration experiment (Appendix C), *it was observed that although the risk of infection changed over time, there is a need for more time studies to be conducted on the guinea pigs to get a better idea of how the risk of infection changes over time.*

The Wells/Riley equation uses an exponential survival distribution that implies that the hazard does not change over time. *A more detailed study may reveal that the risk is in fact not constant over time, which may well provide argument for challenging the Wells/Riley equation.*

The calibration experiment described in this thesis has provided certain results that indicate that these assumptions are questionable. The author intends, with further statistical analysis, using additional data that will be derived from future experiments, to challenge the Wells/Riley equation by introducing the dynamic behaviour of the quanta of infectious particles generated and dispersed within the spaces occupied by infectious patients. *The contribution of this study could have an important impact on future experiments, least of all our understanding of transmission of airborne infection. Prior to the creation, validation and calibration of the AIR facility this would not have been possible* [41] [42].

The sampling capability of the apparatus *will now allow scientific studies to answer fundamental questions about the infectiousness of MDR-TB and the efficacy of various engineering interventions to minimise the spread of airborne disease.* These experiments will provide the scientific blue-prints for design of safer health care facilities and the development of improved building and construction standards for improved infection control to minimize the spread of the airborne disease, particularly in light of the global HIV/Aids epidemic [1] [29].

The consequential contributions of this study are thus:

- *The two-animal exposure chamber facility allows experiments to be conducted in a controlled manner, resulting in direct measurements of the efficacy of interventions against the same human source strength of infectious aerosol.*

- *Unlike most clinical field trials, different interventions can now be compared to each other under identical conditions of host susceptibility (guinea pigs), physical environment (the AIR facility) and human infectious source strength (the 6 patients on the ward).*

- *Moreover, high rates of infection in initial experiments indicate that multiple interventions can be tested sequentially over relatively short time periods compared to most clinical trials.*

- *Variable host susceptibility as well as variable environmental conditions are eliminated in the experimental apparatus design.*

In addition to the above, by taking advantage of the latest advances in molecular biology and genetic engineering, *lessons learned about TB aerobiology and the control of airborne M. tuberculosis can be applied to other agents that have airborne potential, such as measles, influenza, Severe Acute Respiratory Syndrome (SARS) and smallpox, to name just three agents of immediate global concern* [42] [43].

Two conference papers from this thesis, based on chapters 4, 5 and 6, were presented during May 2005 and have been submitted for publication in the international journal of the American Industrial Hygiene Association (AIHA).

Conference papers have been presented at the following meetings:

- The International Society for Environmental Epidemiology (ISEE) Conference, 2005, Johannesburg, South Africa.

- The American Society for Heating, Refrigeration and Airconditioning Engineers (ASHRAE) Summer Conference, June 2006, Quebec City, Quebec, Canada.

- The Expert Consultation meeting on Drug-resistant Tuberculosis hosted by the SA Medical Research Council (SA MRC), the Centers for Disease Controls (CDC) USA, the Global Aids Program (GAP) and supported by the World Health Organisation (WHO), September 2006, Johannesburg, South Africa.

Additional contributions are as follows:

- *Clinical predictors of patient infectiousness (e.g. active coughing, sputum smear-positive disease, cavitary disease on chest x-ray) can now be assessed in addition to the overall duration of infectiousness.*

- *The AIR facility presents a unique opportunity for the evaluation of novel approaches to drug administration (e.g. through inhalation), as well as for the evaluation of new drugs in biological (rather than laboratory) models.*

1.7 Beneficiaries of the study

South African health care workers are increasingly becoming aware of the need to manage infection control for preventing the spread of infectious diseases in health care settings; however; due to inappropriate facility design and the lack of implemented environmental controls, appropriate infection control strategies are largely non-existent, particularly with regard to airborne diseases such as *M. tuberculosis* and drug resistant *M. tuberculosis*.

Proof of the duration of infectiousness of MDR-TB patients would have a major impact in both developing and industrialized countries, reducing the risk of drug resistant *M. tuberculosis* transmission and avoiding prolonged hospitalization of patients.

Of particular relevance for Africa and other resource-limited, high TB-HIV burden settings is the development of evidence-based strategies for appropriate and affordable infection control in congregate settings such as hospitals and prisons.

Moreover, lessons learnt about the aerobiology of *M. tuberculosis* and drug resistant *M. tuberculosis* and their control would also be applicable to other agents with airborne potential, notably influenza, smallpox, bird-flu and SARS. All of these agents are of immediate global concern and measures to limit their spread are urgently required.

1.8 The organization of the study

In addition to the first chapter (introduction) this study consists of six other chapters followed by the references and appendices.

The aim of chapter two is to discuss person to person transmission of *M. tuberculosis* as an airborne respiratory disease, their origins, the theory behind droplets, droplet nuclei and dust. The hypothesis of the contagious potential of droplet nucleus borne infection is developed to derive the Wells/Riley equation of the probability of infection.

Chapter three discusses the need for evidence based engineering interventions to combat the spread of infectious airborne disease. The effectiveness of these interventions must be sought through clinical studies.

Chapter four provides the development of the AIR facility, a unique full scale apparatus which utilises guinea pigs as a biological model to study human-generated (i.e. not artificially aerosolized) airborne transmission and infectivity including the effectiveness of various interventions.

Chapter five details the procedures followed to validate the apparatus. Validation being the documented procedure required for obtaining, recording and interpreting the results needed for assurance and confirmation that the various systems installed for

functional support, health and safety at the facility will consistently perform and comply with a pre-determined specification. The experiments discussed included:

- The experiment to validate the effectiveness and air tightness (leakage factor) of the air distribution from the wards to the animal infection chambers via the "infected exhaust air" transfer duct system.

- The experiment to determine the effective transfer of infectious airborne particles from patient wards to the animal rooms via the "transfer duct" system.

- The experiment to determine the efficacy of the in-duct UVGI units installed in the "transfer duct" system.

Chapter six covers the calibration of the apparatus. The objective of calibrating the apparatus was to demonstrate that its functional requirement; namely its ability to quantify the infectiveness of the ward air; is satisfied. The ability to effectively transfer infectious airborne particles from patient wards (clinical unit) to the animal exposure chambers resulting in infection of adequate numbers of guinea pigs was evaluated.

Chapter seven, in summarising the development and operation of the unique AIR facility, provides positive conclusion to the operation of the apparatus highlighting that it is now possible to undertake air sampling of human generated infectious *M. tuberculosis* using guinea pig air sampling.

The contribution of this study will provide the much needed engineering interventions against the spread of airborne disease to be effectively studied. Recommendations for future studies are also provided.

Finally, there are three appendices which should be viewed as initial extensions of the main theme of the study.

2

TRANSMISSION AND INFECTIVITY: *Mycobacterium tuberculosis*

If diseases are transmitted by air, the organisms which cause them must exhibit some fairly obvious properties. There must be some means by which they can be liberated into the air, they must remain both alive and infectious in transit and there must be some means by which they can again be implanted onto or into the susceptible tissues of a new host. Micro-organisms are particulate bodies of definite dimensions and composition which, when suspended in air, do not diffusely poison the atmosphere but obey the physical laws of small particles in suspension. This chapter provides the theory of the contagious potential for the development of the Wells/Riley equation which defines the effective contact rate.

2 TRANSMISSION AND INFECTIVITY: *Mycobacterium tuberculosis*

2.1 Introduction

In the 1930's Yaglou, working at Harvard School of Public Health [44], introduced occupant comfort as determined by the ability of subjects to detect body odour in an exposure chamber as a scientific basis for satisfactory indoor ventilation. Despite many advances in the understanding of indoor air quality, the comfort of the majority of room occupants remained the primary determinant of adequate ventilation, now incorporating additional parameters such as temperature and humidity.

In recent years; however; health as well as comfort has become a goal of indoor environmental control. Two types of diseases associated with exposure to indoor air have been identified and labelled, namely, the "sick building syndrome" (SBS) and "building-related illness" (BRI).

Person to person transmission of airborne respiratory infections, an important consequence of BRI, could be a potential determinant of adequate ventilation, if only there were objective criteria by which to set a standard. People in buildings frequently report discomfort, BRI and other symptoms, which may be caused by the indoor environment (e.g. sick building syndrome).

The particulate nature of airborne infection has a number of important consequences. First, a particular volume of air is infectious only insofar as it contains a particle capable of giving rise to infection. As a result, the factors which effect the concentration of infectious particles in the air are of importance in determining whether a person exposed to the air becomes infected or not.

This concentration is the resultant of the rate of addition and rate of removal of infectious particles to and from the air. In appreciating this, it becomes apparent that the key to understanding the transmission and control of airborne infection is

contained in the study of the origin, behaviour and means of elimination of infectious airborne particles.

2.2 Origin and numbers of airborne organisms

Organism-bearing particles are liberated into the air by two principal means. Primarily, they arise from activities involving the respiratory tract such as sneezing and coughing as well as from movements which shed bacteria bearing particles from the skin, or from clothing, dressings and so on where they have accumulated from contact with skin or orifices, including wounds.

Secondary, they arise from the re-disturbance of organism-bearing particles which have accumulated in the dust of rooms. Some of these are derived from humans and some from other sources.

In quiet breathing, very few organisms are liberated, but in talking, coughing and especially in sneezing, large numbers of droplets, many of which contain organisms, are ejected. The high velocity of air passing over the respiratory tract tears off myriads of small droplets which are forcibly ejected into the air.

Duguid [45], in one of his series of tests, found that talking with various degrees of emphasis, liberated 0 – 210 bacteria-bearing droplets less than 100 microns in diameter, while various coughs liberated 0 – 3 500 and natural and artificial sneezes between 4 500 and 1 000 000 droplets.

The size of the droplets swept by an air current from the surface of a liquid is determined principally by the velocity of the air and the surface tension of the liquid. As the air velocity increases, the size of the droplets decreases until above 100 m/s where the diameter of the water droplets approaches a minimum of 10 microns. In sneezing and coughing, the peak air flow in the bronchi approaches 300 m/s and consequently the droplets ejected should be about 10 microns in diameter. Duguid [45] concluded that, from direct measurements of the droplets ejected in sneezing, the mean droplet diameter was about 6 microns. In all these observations; however;

marked differences had been observed in the numbers of organisms liberated by different individuals.

Riley [46] argued that unusually large numbers of organisms released may arise from enhanced multiplication or enhanced liberation. These two factors cannot be entirely separated but may be grouped under four headings: type of disease, site of carriage, efficiency of atomization and personal habits.

2.3 Droplets, droplet nuclei and dust

There is therefore a fundamental distinction between infectious particles which remain suspended and those which settle and are removed by sedimentation. This distinction gives rise to the two basic varieties of airborne infection: droplet nucleus-borne and dust-borne.

The larger droplets and mucus fall rapidly to the ground where they become mixed with, and in part adherent to, the heterogeneous mixture of particulate animal, vegetable and mineral debris which constitutes household dust. There they gradually dry and in this state, organisms may survive for long periods to be re-suspended when the dust is disturbed. The characteristic of these particles is that, like the droplets from which they were derived, they settle rapidly after disturbance and form a persistent reservoir of infectious particles in the room. The fate of the smaller droplets is; however; quite different.

After ejection from the mouth and nose, even the smallest droplets begin to fall to the ground, the actual rate of fall being determined by size. However; as droplets present a large surface they tend to evaporate rapidly in the air. (The speed of evaporation will therefore depend on the size of the droplet.)

As droplets fall through the air, they evaporate and those below a certain size fall sufficiently slowly and loose water sufficiently rapidly to evaporate almost instantaneously (this may be shown by serial stroboscopic images of a sneeze). The rapidity with which the evaporation occurs depends on the difference of pressure of

water vapour in the atmosphere and that at the surface of the droplet. It is influenced therefore, both by the humidity of the atmosphere and by the composition of the droplet, since the vapour pressure of a solution is less than that of its solvent. In this way the droplet diminishes in size until the concentration of dissolved substances is such that the vapour pressure which the droplet exerts equals that of the atmosphere.

The residue of the droplet after evaporation, which would contain the organisms originally present when expelled from the respiratory tract, has been called the *droplet nucleus* [47]. Droplet nuclei are so light that they do not settle in the gentlest of moving air of occupied spaces and remain suspended until they are removed by ventilation or disinfecting the air.

Particles in the air may therefore be classified by determining their settling velocity. In still air, droplet nuclei settle at about 0.20×10^{-3} m/s. The droplet nuclei may stay suspended almost indefinitely in occupied spaces where the air is rarely still.

2.4 Contagious potential

Analogous factors are implicated in the introduction of infection in a community; the number of infectious sources, the number of susceptible members of the community and a factor like that of the route of challenge which determines the effectiveness with which the infection is applied to the susceptibles. Infections occur only when contacts are made which are effective in the transmission of the disease. These are referred to as ***effective contacts***.

The above are related to the crowding of susceptibles or *density of susceptibles* (the chance of effective contact is also directly related to the number of infectious cases present). By retrospective analysis of detailed epidemiological data it has been concluded that it is possible (in special circumstances) to derive a constant which defines the ***effective contact rate***. These fundamental factors involved have been related as follows (Soper [48]):

The number of new cases C occurring in each generation of an epidemic is directly proportional to:

- The number of susceptibles S;
- The number of infectors I; and
- The effective contact rate r.

Hence

$$C = rIS \tag{2.1}$$

The above could apply to any form of contagion. That is to say to any disease spread by contact. Respiratory droplets, with their limited flight, range and dependence on the simultaneous presence of source and subject, behave as a form of contact and so, for similar reasons, do droplet nuclei. It was for this reason that Wells called droplet-nucleus-borne infection, ***airborne contagion***.

The predicted pattern of infections arising under the simplest conditions is thus demonstrated and the factor determining the contagious potential is rS (the number of susceptibles multiplied with the contact rate).

The droplet-nucleus hypothesis implies that the transmission of airborne infection within a single enclosed atmosphere follows the above expression, $C = rIS$ and that r depends upon the pulmonary ventilation per susceptible, which in the case of the biological calibration of the AIR facility, being the guinea pigs, the number of infectious particles produced by the infectors (patients) and the ventilation of the room.

2.4.1 Airborne contagion (or Droplet-nucleus-borne infection)

When considering ***airborne contagion***, it should be noted that what is not well understood is how many infectious particles, or droplet nuclei, are required to infect. This number, whatever it may be, will be considered a unit of airborne infection, or a ***Quantum***.

The following assumptions must then be qualified:

1. It is assumed that each quantum breathed by a susceptible S will produce a new case and that the probability of a susceptible S breathing in more than one quantum is negligible.

2. The rate at which quanta I of infection are added to the air by infectious cases will be considered constant; hence differences in infectiousness will be neglected.

3. Since the nuclei disperse rapidly throughout an enclosed atmosphere, it will be assumed that they are evenly distributed.

By assuming therefore that for airborne infection C, I and S keep the same meaning as the above formula (1), $C = rIS$ will still apply.

2.4.2 Epidemiological implications of the droplet-nucleus hypothesis

Droplet-nucleus-borne infections are not associated with epidemiologically important external reservoirs of infection. The infectious particles disperse throughout indoor atmospheres where proximity in time and place between host and victim is required.

It is not enough to deposit infectious material on the surface of the body. In order to establish effective contact, infectious material must be implanted on susceptible tissue within the respiratory tract of a susceptible person. Droplet nuclei are aerodynamically suited and qualified to reach susceptible tissues deep within the respiratory tract.

The particulate nature of airborne infection has a number of important consequences. First, a particular volume of air is infectious only so far as it contains a particle capable of giving rise to infection. As a result, the factors which effect the concentration of infectious particles in the air become of paramount importance in determining whether a person exposed to the air becomes infected or not.

Because the doses of organisms required to induce most human infections are not known, Wells introduced the idea of *quantal infection* [49]. A ***quantum*** of infection is that dose, whatever it may be in terms of numbers of organisms, required to produce infection in a susceptible person.

In order to define the expected contact rate of infection the following will need to be assumed:

1. A single enclosed atmosphere, containing at least one infectious case and several susceptibles;

2. The unit of infectious particles will be a quanta of infection produced by an infector;

3. Each quantum breathed by a susceptible will produce an infection;

4. Differences in susceptibility amongst susceptibles will be negligible;

5. The rate at which quanta of infection are added to the air by infectious cases will be constant;

6. The droplet nuclei are evenly dispersed throughout the enclosed atmosphere; and that
7. The enclosed space is vented at a constant rate and hence the droplet nuclei are vented at a constant rate.

By keeping the above definitions of C, I and S and in addition;

q = the number of quanta, of airborne infection produced by an infector

p = the volume of air breathed by a susceptible

Q = the volume of air vented from the enclosed space in which infectious contact takes place.

In each case the period of time involved is the period of infectious contact.

qI = quanta of airborne infection produced by infectors
qI/Q = quanta produced by infectors per unit volume of air
Sp = volume of air breathed by susceptibles.

Since from the above:

$Sp \times qI/Q$ = quanta of airborne infection breathed by susceptibles
= the number of new cases

Therefore

$$C = \frac{Sp \times qI}{Q} = \frac{pq}{Q} \times IS \quad (2.2)$$

Since p, I and Q are all constant; the quantity pq/Q is a constant. It may be seen therefore that equation (2) has the form $C = rIS$ that the effective contact rate (r) can be defined:

$$r = pq/Q \quad (2.3)$$

Therefore, from (3) the following equation is noted:

$$\textit{Infection rate } (r) = \frac{\textit{Volume of air breathed by susceptibles} \times \textit{Quanta of infectious particles by Infectors.}}{\textit{Volume of air ventilated from enclosed space}}$$

From the above definition for contact rate $r = p \times q/Q$, it is noted that the effective contact rate r is a function of the volume of air breathed and the quanta per unit volume.

As the volume of air breathed by the guinea pigs (susceptibles) may be considered a constant, the varying factor that will affect the infection rate r will be the quanta per unit volume, or concentration q/Q of infectious particles.

2.5 The transmission of *M. tuberculosis*

Tuberculosis, as an infectious disease, is generally transmitted from one person to another through the air. The bacterium, *M. tuberculosis*, becomes aerosolised in small droplets of water or bodily fluid when a person with the disease of the lung coughs, sneezes, laughs or sings. Many of these respiratory droplets dry into "droplet nuclei" and become airborne following room air currents as described in the foregoing paragraphs [49].

Infection can occur when the bacterium or droplet nuclei are inhaled. While many at-risk populations have been identified, those at greatest risk are close contacts, such as healthcare professionals.

M. tuberculosis infection is; however; different from TB disease. People having the infection (without disease) have been infected by the *M. tuberculosis* bacterium. They are not symptomatic because their body's immune system has encapsulated the infectious material in the lung, where it is held dormant. They cannot spread the bacterium or disease to others when in this phase. However; people with the infection may develop the disease in the future.

The infection turns to disease when the body can no longer contain the infectious material in the lung. The infection then spreads, usually within the lung and possibly to other areas of the body. This spread usually shows up as persistent respiratory symptoms such as cough and fever. People with TB disease can therefore transmit it.

2.6 The Wells/Riley equation

In order to handle essential basic variances it is fruitful to employ an appropriate probability distribution that specifies the chance that an individual selected at random will be found to have given measurement, or range of measurements.

Suppose the number (quanta) of infectious particles emitted during a given time interval are counted, for a specified number of pulmonary diseased patients, the

resultant number of infections occurring in a given interval, under specified conditions, the most suitable description is likely to be the Poisson Distribution for which the probability for observing r infections is:

$$e^{-m} m^r / r! \quad (0 \leq r \leq \infty) \qquad (2.4)$$

Where:

r = number of infections (or infection rate described above) and

m = the average of the distribution.

Infectious droplet nuclei are considered to be randomly dispersed in room air. The number of susceptibles S who become infected C is related to the number of cases in the infectious stage I, the pulmonary ventilation (breathing) rate per susceptible, in volume per unit time p, the exposure time t, the removal rate by fresh air (dilution ventilation) Q ***and*** the number of doses of airborne infection q added to the air per unit time by a case in the infectious stage.

As the number of infectious particles required to infect a susceptible is not known, Wells referred to the infectious dose as a quanta q (whether it be a single or several organisms). Any single droplet nuclei may contain quanta of infectious particles.
If infectious droplet nuclei were evenly distributed in a defined space, the ***number*** of quanta inhaled by susceptibles N would be equal to the concentration of quanta in the air times the volume of air breathed by the susceptibles.

In the steady state, the concentration of quanta would equal:

$$Iq / Q \qquad (2.5)$$

(i.e. the number of infectors I, times the rate of production of quanta per infector q, divided by the volume of fresh or disinfected air into which the quanta are distributed Q.) The volume of air breathed by susceptibles would be equal to:

$$Spt \qquad (2.6)$$

(Where the number of susceptibles S, times the pulmonary ventilation rate per susceptible p, times the duration of exposure t.) The number of quanta N inhaled is therefore:

$$\begin{aligned} N &= \text{concentration x volume} \\ &= Iq / Q \times Spt \\ &= S\,(Iqpt/Q) \end{aligned} \qquad (2.7)$$

Where $Iqpt/Q = 1$, N would be equal to S and each susceptible would inhale one quantum of infection. All susceptibles would therefore be infected.

In reality; however; infectious droplet nuclei are separated by large volumes of uninfected air and the distribution of infectious particles are more nearly random than even. And so if $Iqpt/Q = 1$ and the number of airborne quanta inhaled by susceptibles equalled the number of susceptibles, some susceptibles would escape infection and others would inhale more than one quantum.

According to Poisson's law of probability of small chances, the probability of escaping infection in this special case would be approximately e^{-1} (or 0.37), where e is the base of natural logarithms with a value of approximately 2.7.
The probability of acquiring infection would be ($1 - e^{-1}$), or 0.63 and the total number of infections would be $S(1 - e^{-1})$.

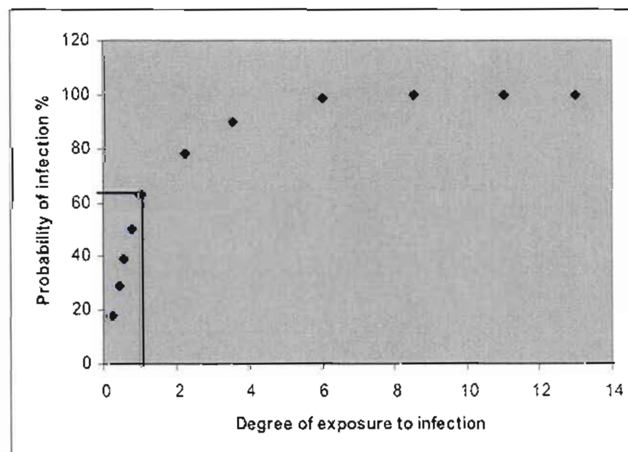


Figure 2. 1: The probability of infection ($1 - e^{-Iqpt/Q}$) as a function of degree of exposure to infection Iqpt/Q. (The point where Iqpt/Q =1 is identified by the co-ordinates)

Thus, in this special case where $Iqpt/Q = 1$, 63% of susceptibles would be infected with random distribution as apposed to 100% with even distribution.

The general expression, based on these physical relationships is therefore:

$$C \quad = S(1- e^{-Iqpt/Q}) \tag{2.8}$$

Where C = the number of new infections or cases. In the above expression, the exponent $Iqpt/Q$ represents the degree or intensity of exposure in infection and therefore the:

$$\textit{Probability of infection} = C / S = (1 - e^{-Iqpt/Q}) \tag{2.9}$$

This is known as the *Wells/Riley* equation [50].

When considering equation (2.3), $r = pq/Q$ where p, q and Q are considered constants for a given set of susceptibles housed in a common space with constant air flow rate from such a space, the Wells/Riley equation may be written as:

$$C / S = (1 - e^{-Irt}) \tag{2.10}$$

where

$$- Irt = ln\, [(S-C)/S] \tag{2.11}$$

When considering the logarithmic power function, it follows that:

$$Irt = ln\, [S/(S-C)] \tag{2.12}$$

In terms of ***effective contact rate*** therefore:

$$r = \{1/It\}\{ln\, [S/(S-C)]\} \tag{2.13}$$

By using the sample data from the calibration experiment discussed in Chapter 6, the equation was investigated to determine if the assumptions made in deriving the equation (2.10) above apply. This investigation is presented with conclusions in Appendix D.

2.7 Infectivity and virulence of airborne *M. tuberculosis*

Certain strains of organisms are responsible for outbreaks of infection much more than others. This is sometimes taken to result from differences amongst strains in their capacity to establish themselves in a new host (Infectivity), or to produce progressive disease (Virulence).

The experimental demonstration of such differences between strains is much influenced by the dose of organisms, route of challenge and host resistance.

Theoretically, organisms discharged into the air and inhaled in a droplet nucleus may arrive in contact with the host's tissues:

1. Alive, infective and virulent;
2. Alive and infective but anti-virulent;
3. Alive but non-infective; and
4. Dead.

As a final possibility, since "alive" means recoverable in culture and the susceptible tissues of a host may provide more favourable conditions for multiplication than artificial media, we must add:

5. Infective (and virulent) but "dead".

We are not yet technically in a position to make these distinctions in practice for *M. tuberculosis* in particular, but they can be explored experimentally by exposing animals in suitable chambers.

2.8 Conclusion

Factors governing the chance of infection and the chance of non-productive exposure are considered in terms of the likelihood breathing a quantum of airborne infection. Because of the tendency of dust to settle, dustborne infection is associated in time with places. It need not be associated in time with occupancy by an infectious case because settled dust constitutes an external reservoir of infection.

Because of the aerodynamic characteristics of dust, dustborne infection, when inhaled, is ordinarily trapped in the upper respiratory tract.

Droplet-nucleus borne infections are not associated with epidemiologically important external reservoirs of infection. The infectious particles disperse throughout indoor atmospheres and are removed either by dilution ventilation or by air disinfection techniques. Proximity in time and place between host and victim is required.

Droplet nuclei are aerodynamically suited to reach susceptible tissue deep within the respiratory tract and only by exposing animals in suitable chambers can viable or non-viable organisms be defined.

The Wells/Riley equation, as presented in this chapter, has been developed on the assumption that the droplet nuclei are randomly dispersed within a defined space and that all conditions are in a steady state condition. In reality; however; this may not be the case. The results from the various experiments to be conducted at the AIR facility may well challenge the hypothesis on which this equation has been based.

3

THE NEED FOR EVIDENCE BASED ENGINEERING INTERVENTIONS TO COMBAT THE SPREAD OF INFECTIOUS AIRBOURNE DISEASE

Like ventilation, the precise application of UVGI and other novel interventions needed to minimize airborne transmission is still part science and part art. Confirmation of the effectiveness of these interventions ***still*** *needs to be sought through clinical studies.*

3 THE NEED FOR EVIDENCE BASED ENGINEERING INTERVENTIONS TO COMBAT THE SPREAD OF INFECTIOUS AIRBOURNE DISEASE

3.1 Introduction

The concerns of healthcare providers in high-prevalence situations must be answered by effective control strategies, including both administrative and evidence based engineering controls to reduce transmission.

Health care facilities in particular, comprise complex and diverse environments, often consisting of technically complex and specialized sections. The public interface of health facilities (e.g. outpatient areas or casualty) need to be designed and built according to clinically acceptable standards.

The planning, design and management of health facilities is more often than not, completely detached from the intended function, with the emphasis on aesthetics and comfort, rather than on functionality and fundamental principles to ensure a safe environment and adhere to essential requirements for infection control.

The practice of providing natural ventilation for infectious disease hospital settings is the cause of grave concern to many engineering practitioners and medical researchers worldwide. The belief by certain designers that by simply following the minimum requirements for window openings as prescribed in the NBR will suffice in containing minimum contaminant levels such as CO_2, etc., or ensure satisfactory thermal conditions within the building, is naive.

Without sophisticated computational flow dynamic (CFD) studies of the specific building model, it cannot be assumed that the opening provided will, in fact, provide the airflows within the building spaces being designed.

Further, by simply accepting these minimum standards, the reality of seasonal change is ignored due to the obsession with thermal comfort. These window openings would surely be closed during midwinter periods and in particular after dark.

Although many of the public health buildings in South Africa may be classed as aged, there is a perception that these buildings are ideal for preventing the transmission of TB since they have high ceilings and long corridors, with plenty of open space for air to be transported. There are also many windows to allow the unrestricted flow of air through the building and sunlight into the building.

This perception was probably correct when these buildings were originally designed according to the above standards/norms, but current realities are different. In hospitals which treat TB, especially those having MDR-TB wards, it is not uncommon to find that:

- The corridors are packed with patients awaiting treatment and in some cases with beds that cannot be accommodated in overcrowded wards.

- Overcrowding within the building, in which air movement is restricted, creates a more "close and humid" environment.

- Doors and windows are normally kept closed at night to create a more stable environment for sleeping and for security.

Due to physical constraints, in many cases, aged facilities could not accommodate central heating and ventilation systems without major alteration to the building fabric.

The tendency has therefore been to install ceiling fans and/or side-wall heating fans in patient rooms to address the issue of thermal discomfort. Unfortunately, this arrangement provides no "control" for preventing the transmission of airborne infectious organisms within the facility. Ceiling, or "side-wall", heater-fan units merely cause air mixing rather than dilution ventilation by fresh air supplied via a mechanical ventilation system.

Every health-care facility should, as part of its overall infection-control program, have a TB infection-control program in place. Based on the risk assessment for the facility, proper administrative, environmental and respiratory protection policies and measures to prevent health-care associated transmission of *M. tuberculosis* should be adopted. These policies should form the basis for a facility-specific TB infection-control program. Administrative controls being the most important part of the TB infection-control program.

The specific details of the TB infection-control program will differ for each facility, depending on whether patients with suspected or confirmed TB disease might be encountered in the facility or whether patients with suspected or confirmed TB disease will be triaged or transferred to another health-care facility. Each facility design will influence its functional and utilisation possibilities, which in turn will also influence the TB infection-control program details.

Appropriate, innovative evidence based engineering controls need to be designed for resource-limited high-prevalence countries.

3.2 The need to establish the efficacy of various engineering interventions

Among the important questions that urgently require answers are:

1. Is it possible to create germ-free breathing zones in patient rooms (displacement ventilation), or is better air mixing within rooms the more protective strategy?
2. How effective are the various engineering interventions that may minimize the spread of the disease.

In addition to these important control issues, there are crucial basic questions about the infectiousness of patients with MDR-TB and HIV. There are several reasons why this is a critical time to undertake the research on transmission and recommended environmental controls:

1. There is great concern about the very real possibility of TB transmission among institutional staff and administration. Currently, there is no effective chemo prophylaxis regimen for those infected with drug resistant *M. tuberculosis*.

2. The costs of the interventions currently recommended to prevent institutional TB transmission are extremely high, out of reach for most developing countries where the reliable supply of essential antibiotics is often limited by financial constraints.

Moreover, there are conflicting views; and little hard evidence available; on efficacy or cost effectiveness of various interventions. Although some experimental studies of air disinfection are underway, the experimental facility is a unique international laboratory for the study of TB transmission and air disinfection through a variety of potential interventions.

Due to the high capital outlay required, medical facilities in countries such as South Africa; which would be defined as developing; generally do not have ventilation systems specifically installed to achieve the dilution levels that may reduce the risk of airborne particle exposure.

Further, ventilation that might be protective for a brief exposure to an airborne agent of ordinary virulence might not be protective if exposure were to be prolonged or if the airborne agent were highly virulent. Quantifying the infectiousness of an average case of TB, or influenza, could be used to develop a useful ventilation standard.

Ventilation could be recommended to handle the "worst case" scenario, the highly infectious case, which, however uncommon, is particularly hazardous. However; recommending very high ventilation in the vast majority of situations would be impractical, resulting in high capital cost and in most cases be wasteful of energy, with possible discomfort for room occupants.

The solution to the above economic constraint may therefore be air disinfection rather than dilution, as it need not rely entirely on mechanical ventilation.

The air hygienist, William Firth Wells [51], a contemporary of Yaglou's at Harvard, who conceptualised droplet nuclei transmission, also recommended: "Ventilate for comfort, but irradiate for infection control".

Wells's prophetic remark was referring to the use of upper-room ultraviolet germicidal irradiation, UVGI, which he found to be an effective means of sterilizing room air.

Air disinfection by means of UVGI could effectively reduce the amount of air changes to a space and save energy. Such air disinfection may be the single most cost-effective engineering intervention under circumstances where air circulation is an important transmission factor.

Although far less familiar to most engineers than ventilation, UVGI is an increasingly well-defined and accepted air disinfection technology. Guidelines for its possible use have recently been published in ASHRAE transactions. Most publications qualify these guidelines as being dependent on still to be proven efficacy.

In order to implement appropriate TB infection control programmes and functional methods for in-house risk management to minimise the spread of the infectious airborne disease in the wide variety of healthcare facilities that serve moderate to high TB prevalence communities, research into and the development of evidence based interventions are sorely needed.

Little is known about drug resistant *M. tuberculosis* transmission, infectivity and the efficacy of necessary interventions for infection control, particularly in light of the global HIV/Aids epidemic.

Despite strong evidence on the efficacy of various engineering interventions against TB, there have not yet been any clinical trials on prevention of *M. tuberculosis*

transmission – in particular there have been no trails of ventilation, filtration, UV irradiation or respirator use for TB control purposes

The AIR facility creates a unique opportunity to conduct research on robust and affordable environmental and engineering controls to reduce airborne transmission, such as natural ventilation (e.g. open windows) and air disinfection (e.g. upper room ultraviolet germicidal irradiation UVGI).

3.3 Engineering interventions for environmental infection control

In its *Guidelines for Preventing the Transmission of Mycobacterium Tuberculosis in Health-Care Facilities*, published in 1994, the US Centers for Disease Control (CDC) [28] indicate that a tuberculosis infection control programme should be based on a hierarchy of control measures.

The second level of the hierarchy, as noted above, is the use of engineering controls to prevent the spread and reduce the concentration of infectious droplet nuclei. These controls include:

- Direct source control using local exhaust ventilation.
- Controlling the airflow within buildings to prevent contamination of air in areas adjacent to the infectious source, using contaminant source isolation techniques when designing appropriate ventilation systems.
- Dilution and removing contaminated air via controlled (forced) ventilation systems.
- The removal of contaminants from the air via filtration.

The CDC [28] allow the use of portable High Efficiency Particulate Arrestance (HEPA) filter units in tuberculosis isolation rooms as a means of achieving the desired

air change rate for the occupied space, thus augmenting the mechanical ventilation system.

The above are discussed in more detail in the sections that follow.

3.3.1 Direct source control using local exhaust ventilation

Local exhaust systems capture and remove airborne contaminants at, or near, their source before they are dispersed into a space. Local exhaust ventilation is preferable over dilution ventilation as a means to control exposure to airborne contaminants, because exposure is much less likely to occur.

Local exhaust ventilation systems are designed for specific applications, or in conjunction with dilution systems. A system typically includes a hood or localised extract point, ductwork, an air cleaner and a fan. The localised extract point is the most important part of the system. This extraction point should be as close as possible to the source of the contamination. Air is withdrawn at a rate sufficient to ensure that the contaminant released is entrained in the air stream moving into the localised extract point, or that the least amount of contaminant escapes into the general room air.

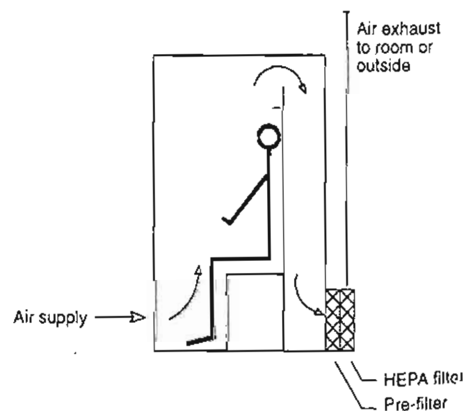


Figure 3. 1: Operation of a typical containment booth for local exhaust ventilation

It is not feasible; however; to design local exhaust ventilation systems for every TB patient. The source of contaminant can therefore not be controlled by local exhaust ventilation in ward situations.

3.3.2 Containment source isolation by appropriate ventilation systems

Containment source isolation refers to preventing contaminated (infected) air transported from one occupied building zone into another. This involves establishing airflow and air pressure differentials between different zones within the building in order to contain the migration of infected air.

This is extremely difficult to achieve when certain areas of the building have natural ventilation.

3.3.3 Dilution ventilation

Contaminants are removed from indoor air by a building's mechanical ventilation system either by local exhaust ventilation, as described above; or via dilution ventilation. Dilution ventilation is also known as general ventilation.

The term "dilution ventilation system" will be used in this dissertation to describe mechanical contaminant control systems. "Dilution exhaust systems" lower the concentration of contaminants by mixing contaminated air with fresh air, but never reduce or eliminate the total amount of contaminants released.

Dilution occurs when contaminants released into a space are allowed to mix with the outdoor air supplied to the space by the mechanical ventilation system. Sound industrial hygiene practice, as called for in the South African Occupational Health and Safety Act, Act of 1993 [52], dictates that dilution ventilation should be used to control exposure to occupants only if the following criteria are met:

- Small quantities of contaminants are released at uniform rates.

- There is sufficient distance between the worker and the source of the contaminant.

- The contaminant has a low toxicity, i.e. ≤ 100 ppm.

- No air-cleaning device is needed to collect (i.e. remove) contaminants before the exhaust air is discharged to the outside environment.

For comparison purposes, let us liken the criteria above with the situation of a TB patient, hospitalised in an isolation room:

- The TB patient is the source of the contamination.

- The contaminant is the tubercle bacilli expelled by the patient during coughing, singing or speaking. It may be true that relatively "small" quantities of *M. tuberculosis* bacilli may be released during coughing, singing or speaking. Tubercle bacilli may also be generated at uniform rates by the same patient while coughing, singing or speaking, but these events are episodic. In other words, TB patients are not continuously generating droplet nuclei of *M. tuberculosis* bacilli.
- Healthcare professionals delivering care directly to the TB patient within the isolation room are likely to be close to the patient when he/she begins to cough or speak where the instantaneous airborne concentration is higher than the room average concentration.

- The term "infectivity" applies here rather than toxicity. On the sole basis of infectious dose, MTB is a highly infectious organism and as only one, or a few, droplet nuclei are required for infection, air from TB patient wards must be treated to remove droplet nuclei.

Dilution ventilation can be used to *reduce over time* the room average concentration of droplet nuclei in an isolation room generated intermittently by patients.

To this end, the US Centers for Disease Control (CDC) [28] have stipulated that a characteristic of an effective tuberculosis infection control programme includes the design of patient isolation rooms which must achieve at least 12 air changes per hour (ACH) in new or renovated facilities when designing general ventilation systems.

The theoretical process of Dilution Ventilation, Filtration and Ultraviolet Germicidal Irradiation is provided in Appendix A.

However; the CDC note in their guidelines that the effectiveness of the above rate of ventilation in reducing the concentration of droplet nuclei in the room has not been directly evaluated.

The theory of dilution ventilation provided the basis for the design of the transfer of the airborne pathogens from the in-patient wards to the Animal Exposure rooms at the AIR facilities apparatus.

3.3.4 Filtration

Filtration has long been used as an effective means of air cleaning. Air filters remove particles by five known mechanisms, which include:

1. Interception
2. Internal impaction
3. Diffusion
4. Gravitational settling
5. Electrostatic attraction

Filtration efficiency and the reciprocal penetration (arrestance) depend on the size of the particles in the air stream passing through the filter as well as the velocity. The particle size of maximum penetration (i.e. minimum efficiency) is generally accepted as 0,3 µm aerodynamic diameter but may be lower [53].

Aerodynamic diameter is the diameter of the unit density sphere that has the same settling velocity as the particle. Aerodynamic diameter is the standard for not only the shape, but also for the density. Particles with larger and smaller than most penetrating particle size will be removed with greater efficiency. A grading system based on these two performance criteria has been introduced by Eurovent (European Committee of the Manufacturers of Air Handling Equipment) and is shown in the table below [54].

Table 3. 1: Eurovent filter grades

Filter Grade	Average Arrestance (A_m)%	Average Efficiency (E_m)%
G1	$A_m < 65$	–
G2	$65 \leq 80 \leq A_m$	–
G3	$90 < A_m < 80$	–
G4	$A_m \geq 90$	–
F5	–	$40 \leq E_m < 60$
F6	–	$60 \leq E_m < 80$
F7	–	$80 \leq E_m < 90$
F8	–	$90 \leq E_m < 95$
F9	–	$E_m \geq 95$

In addition to the nine grades of filter described, there is another classification known as High Efficiency Particulate Arrestance (HEPA) filters, sometimes referred to as absolute filters. In the CDC guidelines, HEPA filters are defined as air-cleaning devices that have demonstrated and documented minimum filtration efficiency of 99,97% of particles $\geq$ 0,3 µm in diameter.

The International Environmental Sciences (IES) more rigorously defines a HEPA filter as a throw-away extended-media dry-type filter in a rigid frame with a minimum particle collection efficiency of 99,97% for $\geq$ 0,3 µm thermally generated dioctyl phthalate particles (DOP), or specified alternative aerosol and a maximum "clean-filter" pressure drop of one-inch water column when tested at rated air-flow capacity [55].

HEPA filters can be used in a number of configurations, including the following:

- In booths or enclosures recirculating air back into a room.
- Within a room either in a free-standing (portable) air cleaning unit or in wall-mounted units that recirculate air within the room.
- In HVAC ductwork prior to recirculation to other areas, or in ducts prior to exhausting the air to the outside.

3.3.5 UVGI air cleaning (disinfection)

Many medical facilities in South Africa generally do not employ mechanical HVAC systems. In the absence of centralised ventilation systems, natural ventilation via opening windows is used. Unfortunately, natural ventilation provides unpredictable control in preventing the transmission of infectious airborne diseases. Disinfection by ultraviolet irradiation of the upper room may prove to be the best low-cost solution available for South African healthcare facilities.

Unlike odour and temperature control, functions for which ventilation and other forced-air systems are designed, the removal of diluted air still contaminated with infectious particles may require extremely large ventilation rates for adequate protection against hazardous biological agents as noted previously. In most South African healthcare facilities such high volume dilution ventilation may not be feasible. Its efficacy is limited by engineering constraints as well as by cost and comfort conditions.

Disinfection of the exhaust air from a room with the contaminant source offers little protection for persons sharing the same room with a patient who has infectious TB.

Room by room "air disinfection"; however; may provide the greatest benefit. It has the potential of reducing convective transmission within the room and reducing the chance of recirculation within the building. The approach suggested for disinfection

by ultraviolet germicidal irradiation (UVGI) for air disinfection in healthcare settings would be the upper-room irradiation process [56].

UVGI damages the deoxyribonucleic acid (DNA) of microbial cells, killing them outright or preventing their multiplication [57]. As is the case for radiations of all types (ionising and non-ionising alike), the degree of damage to the microbial cell is directly related to the quantity of energy delivered to susceptible cell structures. A consideration of these limits is important because insufficient energy transfer will cause no damage to a microbe, or perhaps permit the cell's reparative mechanisms to overcome the effects of absorbed UV at a rate adequate to maintain viability (whereas excessive energy transfer would be wasteful and unnecessary human exposure to irradiation should be avoided).

The total amount of energy an airborne micro-organism is exposed to is the product of the UVGI intensity irradiating the microbe (expressed in $\mu W/cm^2$) times the period of exposure in seconds. This exposure rate is therefore expressed in $\mu J/cm^2$.

Some microbes are more resistant to UVGI than others. The microbe Mycobacterium tuberculosis (M-TB) requires a greater exposure to UVGI than most bacteria and viruses for death or inactivation [57]. Susceptibility to UVGI has been measured for a number of different species of bacteria and viruses, but because drug resistant *M. tuberculosis* is both an important human pathogen and one of those most resistant to UVGI, it is frequently used as a reference organism when considering UVGI exposure requirements for practical applications to limit transmission of respiratory disease in enclosed spaces.

However; it is neither practical nor realistic to consider UVGI exposure in terms of every single cell dying because individual cells, even from the same culture, show variable resistance to unfavourable conditions. Therefore; it is customary to measure the fraction of the exposed cell population that survives an exposure to a carefully measured UV dose and to make incremental measurements so as to develop dose response curves; a spectrum of survival rates versus exposure. Figure 3.2 shows data points and a smoothed result for *M. tuberculosis*.

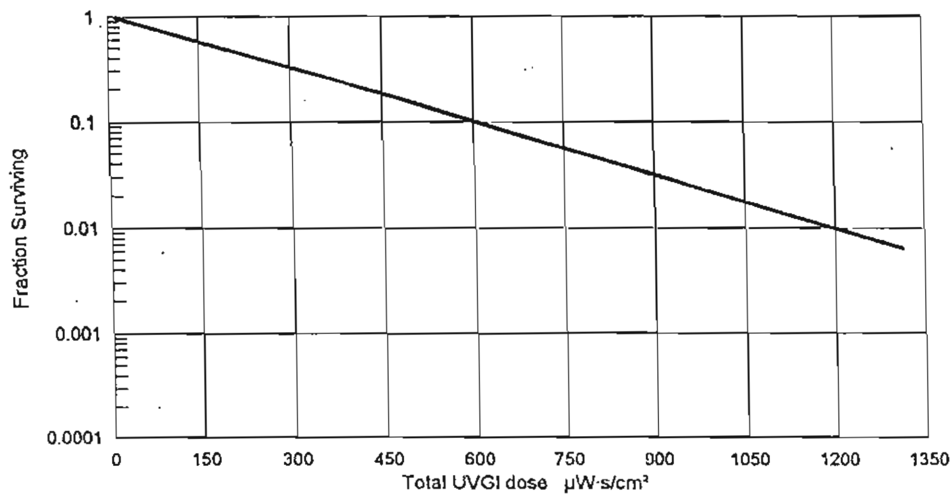


Figure 3. 2: Survival of Mycobacterium (H37Ra) [57]

Upper-room UVGI in particular is of interest for air disinfection. It is employed to inactivate the tubercle bacilli droplet nuclei in the upper room/area, while minimising the ultraviolet exposure to persons in the lower portion of that room. UVGI fixtures are suspended from the ceilings or mounted on walls [57]. The lamp fixture is equipped with louvers to direct the radiation horizontally and away from the lower part of the room. In this way the entire cross-sectional area of the upper room is utilised for air disinfection at a height above head level.

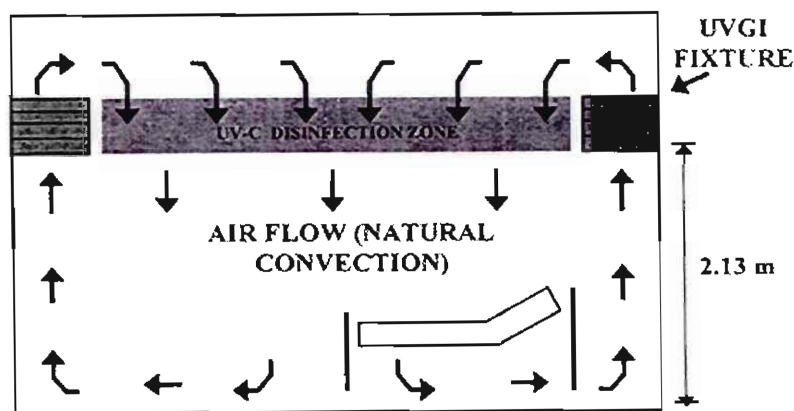


Figure 3. 3: UVGI upper-room irradiation

Natural convection currents, generated by body heat and occupant motion, normally provide air exchange rates between the upper and lower section of the room that may exceed room air exchange rates achieved by mechanical ventilation systems.

It has been suggested that air disinfection by means of UVGI can effectively reduce the amount of air changes to a space and save energy. Should this be substantiated, air disinfection may then be the single most cost-effective engineering intervention under circumstances where air circulation is an important transmission factor [28].

The installation of room air disinfection by UVGI, if proven effective, could provide an appropriate low-cost solution to achieve acceptable indoor air quality levels in healthcare facilities in South Africa. "Air disinfection" by UVGI, in healthcare facilities where drug resistant *M. tuberculosis* transmission can occur from unsuspected cases (such as congregate areas, namely corridors and common rooms) may provide the one most important solution for minimising the spread of the disease.

The reason for this is that these areas serve as conduits for contagion generated by persons with unsuspected disease anywhere in the facility as such spaces are not well suited for dilution by ventilation or forced-air filtration machines.

3.4 Conclusions

Complete room "*M. tuberculosis* removal"; however; cannot be accomplished by dilution air ventilation alone. Its efficacy is limited by engineering constraints as well as by cost and comfort conditions. Unlike odour and temperature control functions for which ventilation and other forced-air systems are designed, the removal of diluted air (still contaminated with infectious particles air) requires extremely large ventilation rates for adequate protection as noted above.

The use of filtration as a means of removing hazardous infectious particles is restricted to "in-room" filtration, as "in-duct" filtration, will not lower the concentration of infectious airborne particles in the occupied zone in which they are being expelled by infectious patients.

Filtration is therefore an effective extension to the dilution process provided by controlled ventilation and can be used as a means to increase the effective air change rate of the ventilation system (at the cost of increased energy consumption).

Filtration as an effective air cleaning process needs to rely on "forced" ventilation as the "driver" to move the air stream through the filtration media in order to overcome the resistance of the media.

Upper-room UVGI technology may prove to be an economical substitution for installing costly ventilation systems in healthcare settings as an effective means of improving air hygiene and reducing communicable airborne disease transmission.

The theoretical process of dilution ventilation, filtration and ultraviolet germicidal irradiation is discussed in more detail in Appendix A.

Proposed guidelines for the design and installation of upper-room UVGI systems have been published, supported with strong evidence from research favouring UV efficacy against TB. The published results of such studies are scant, covering research that has utilised modelling techniques to provide such evidence.

Despite some field trial evidence for its efficacy against other infections, there have not yet been any clinical trials on prevention of TB transmission. Confirmation of the effectiveness of upper-room UVGI must be sought through controlled laboratory studies.

By using the special AIR facility described in this thesis, human-generated airborne transmission (i.e., not artificially aerosolized) can be quantitatively studied and the effectiveness of various interventions measured. The author and members of the international research team associated with the AIR facility are unaware of another airborne infection where human sources of infectious aerosol are continuously available and where quantitative air sampling methods exist to rigorously test control interventions.

4

THE DEVELOPMENT OF THE UNIQUE FULL SCALE APPARATUS

By utilising guinea pigs as a biological model in the development of the unique Airborne Infections Research (AIR) facility (apparatus), human-generated (i.e., not artificially aerosolized) airborne transmission and infectivity can now be quantitatively studied and the effectiveness of various interventions measured.

4 THE DEVELOPMENT OF THE UNIQUE FULL SCALE APPARATUS

4.1 Introduction

As a full scale apparatus, the Airborne Infection Research (AIR) facility now operational, serves as the primary apparatus for the research projects covering various unique studies into *Mycobacterium tuberculosis* transmission undertaken by the International Consortium, namely the SA MRC, CSIR , CDC and Harvard University in collaboration.

The AIR facility is intended for identified research projects intended to provide the necessary scientific evidence of transmission and infectivity of *M. tuberculosis*. In addition, the efficacy of various interventions required for infection control can now be investigated. The AIR facility as an apparatus operates by extracting the infectious air from patient wards and transferring this pathogen contaminated air to exposure chambers housing guinea pigs, which serve as quantitative samplers of the human-generated infectious aerosols as shown in Figure 4.1.

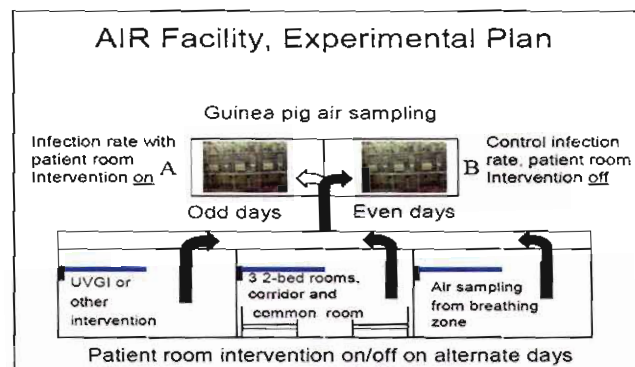


Figure 4. 1: Schematic of the AIR facility experimental plan for testing of infection control interventions on experimental TB ward

Measuring the number of guinea pigs infected over time, linking guinea pig infections to individual patients by means of molecular techniques provides for the unique sampling strategy hypothesised in chapter 1 of this study.

4.2 The design development for the AIR facility as an apparatus

Prepared initially for the Harvard School of Public Health, Harvard University, Boston, Mass., USA; the original sketch plan (conceptual design) developed in January 2001, provided the requirements for the proposal for an apparatus to become the AIR facility at the then HJE Schultz, SANTA (MDR) Centre, later to become the Mpumalanga, MDR-TB referral hospital, situated in Witbank, South Africa.

The sketch plan report provided the design development proposal for the architectural and engineering systems, which met the research requirements for the AIR facility, but also ensured compliance with the design criteria in terms of performance and reliability to satisfy the proposed scientific research projects.

The proposal addressed all aspects of the facility including the essential component of the apparatus namely the HVAC plant. The HVAC provides the required ventilation and temperature (including humidity) control; ensuring minimum compliance to statutory regulations and ASHRAE standards and also transfers the infected air from the wards to the animal exposure rooms.

4.2.1 Requirements identified by the international team in 1991

The requirements for the design were as follows:

1. The facility was to be designed to operate so that the 1951-1962 Riley, Mills *et al* [46], studies on the infectiousness of air from a tuberculosis ward, could not only be continued, but essentially improved upon via the use of more advanced technologies. This in particular with reference to the air transfer system between the wards and animal exposure rooms.

2. The design intent of the animal exposure chamber had to satisfy the scientific rigors of the proposed research and ensure compliance with the minimum ethical requirements for the care and use of laboratory animals.

3. Good animal management and human comfort and health protection required separation of animal facilities from personnel areas, such as the laboratory, overnight and ablution areas. The demands of animal husbandry had to be considered. The issues that required specific attention were food, water, bedding, sanitation, waste disposal and pest control.

4. Building materials that would facilitate efficient and hygienic operation of the animal facility needed to be selected.

The best solution with respect to the design of the installation, the operating parameters and the control strategies of the HVAC plant, to ensure compliance with the research requirements and still satisfy expected scientific rigor, needed to be provided.

4.2.2 Design constraints

The proposed AIR facility was to be housed in an extension to the hospital MDR-TB Ward C.

The ward, with animal rooms, laboratory and in-patient (clinical area) with ablutions, were to be designed to best fit within the overall hospital building envelope.

In order to achieve reliable operating and maintenance practice and to ensure low noise levels from the HVAC equipment, the main air handling plant, chilled water generating equipment, electrical power and electronic control equipment were housed in a separate services building, adjacent to the hospital building. This ensured least disruption to the ward and animal facility by maintenance personal.

4.2.3 Design criteria

Technical: The HVAC system was required to provide ventilation without disruption to the wards and ensure the necessary pressure cascading between the rooms within the facility as prescribed.

In order to satisfy the various experimental needs, the design needed to ensure that the maximum quanta of infectious particles present in the patient wards, is transferred via the air transfer system, at various flow rates, to the exposure chambers. This is essential to simulating exposure of animals as if they are housed within the wards.

In addition to the transfer of the air, the control of temperature, humidity, air velocity and air pressure within the animal rooms are essential. Guinea pigs are extremely susceptible to respiratory disease and a well-designed system is necessary to provide the environmental stability requisite for respiratory disease control.

Issues of concern were:

- Special attention needed to be given to ensuring that equal amounts of air is injected into each cage, to ensure equal exposure of all animals to infectious air from the wards.
- Air velocity (i.e. draft free and even distribution of air to each of the animal cages).
- Air changes per hour (fresh air).
- The heat dissipation from the animals.

The temperature and humidity control equipment needs to maintain, at all times, the selected operating set points in all sensitive areas - wards and animal rooms.

Quality: Equipment selected needed to be of high quality and be reliable. The equipment should be available on the local market with proven record on sales and technical back up service.

Maintenance: The operating vision of the research team was formulated during the final design process (and prior to each research project), therefore ensuring that the commissioning, operating and maintaining of the equipment provided the best option for equipment selection to meet the required scientific rigor of the research work.

Operating: The proposed design needed to result in a product that allowed for various system operating strategies. Each of the envisaged experiments required different strategies with respect to variable air flow through the transfer system, room air dynamics and temperature/humidity control.

The control and monitoring of the apparatus needed to be via a web based Direct Digital Control (DDC) system incorporated into a facility building management system. The controls needed to be accessible for monitoring by all research members of the International research team.

Economics: The use of South African manufactured products, if available, was to receive priority in order to avoid excessive transport and import cost penalties. The design intent needed to consider the running and maintenance costs of the facility when selecting equipment and system configuration.

4.3 The HVAC installation - an integral component of the AIR facility apparatus

The design of the HVAC system for the facility, in particular the air transfer system from the wards to the animal exposure rooms, is the critical component to support the various studies that are to be undertaken. Of equal importance is the design, operation and air dynamics of and within the animal exposure chambers.

The design of ventilation; control systems for hazard control; control of infection and disinfection within the ward; design, construction and operation of the special animal exposure chambers were all essential in preparing for the major objective.

This objective was to determine whether the animals can be infected by breathing the air from the ward when it is occupied by human patients with open tuberculosis and whether such air-borne infection, if achieved, can be eliminated by the various interventions being researched.

When considering the air distribution system from the wards to the exposure chambers, cognizance of the operating parameters of the system used in the1951-1962, Riley, Mills *et al* [38] [39] [40] [41] [46] studies should be taken. These were:

1. The estimated length of the exhaust duct between the patient rooms and the animal rooms was 25m (it is assumed that this was the equivalent length of the exhaust duct work between the rooms and did not allow for duct work connections to exhaust grilles in patient rooms nor to the exposure chambers within the animal rooms).

2. The Riley/Mills apparatus used a wall mounted axial type fan to create negative pressure within the exposure room; thus "pulling" the air from the patient rooms.

3. The air velocity within the exhaust duct was slow; approx. 0.3 m/s.

4. During the 1951-1962 study, the amount of ward air through the exposure chambers and then to the outside was only 108.5 L/s. This was for the exposure to 240 animals (i.e. this equating to 0.45 L/s per animal). Fresh air entered the patient rooms at the same rate as the above exhaust air.

When considering the above operating performance of the Riley/Mills [38] [39] [40] [41] [46] apparatus, the rates of flow per animal for the AIR facility would have been

too low, as the flow rate to the exposure chambers needed to equate to the required ventilation air change rate within the facility (patient rooms, lounge and passage).

In order then to achieve the correct ventilation air flow rate within the patient areas, relative to the exposure chambers and hence max flow per animal without causing draft and discomfort, a deviation from Riley/Mills apparatus flow rates was needed.

Further, as duct dimensions are a function of the air flow and air speed, the Riley/Mills air flow rates would have resulted in excessive duct sizes.

The AIR facility transfer duct design velocities decided upon were:

- Max supply air duct velocity: 5 to 8 m/s
- Minimum exhaust air (patient rooms to animal chambers): 3 m/s

The control of air flow is by control of fan speed, which will operate to satisfy either maximum flow, or pressure, as required by the air distribution system to ensure correlation of air quantities between supply-air and exhaust systems. The foregoing is dependent on the research protocols for ward air change rates, which will be determined for each study.

The exact replication of Riley's ward and exposure chambers in terms of spatial relationships (i.e. having the exposure chambers directly above the wards) was therefore not possible.

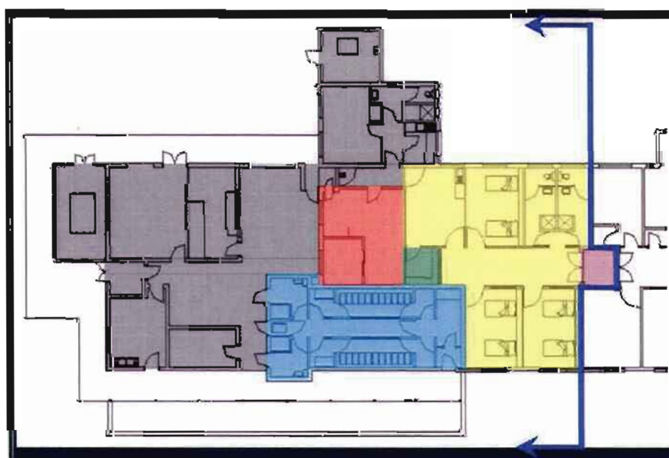


Figure 4. 2: The AIR facility layout showing; in-patient wards (yellow)*, nursing station* (green)*, air lock* (purple)*, animal exposure chambers* (blue)*, laboratory* (red) *and engineering/support service rooms* (grey)

On review of the final solution, when considering the deviations from Riley/Mills apparatus, the relationship between the AIR facilities patient area and exposure chambers within the animal rooms, is an improvement.

The Riley/Mills apparatus used a wall mounted axial type fan to create negative pressure within the exposure room, thus "pulling" the air from the patient rooms. This configuration is not satisfactory as the probability of uncontrolled infiltration of air into the system; due to the negative pressures within the ductwork and surrounding spaces in the exposure rooms; is high and would result in dilution of the air from the patient rooms.

The air transfer system between the wards and the animal exposure rooms at the AIR facility is shorter and air movement is achieved via an in-duct variable speed fan. This provides for better control and allows variability in transfer rates that may be required to satisfy the various experiment research strategies.

Statutory requirements, ASHRAE recommendations, the limitations of animal health and comfort as well as good design principles to ensure correct operation of the systems concerned were considered during the final design of the AIR facilities air transfer system.

In addition to the above arguments for improving the operability of the AIR facility over the original Riley/Mills apparatus, the critical air transfer system design required a more scientific approach.

In order to ensure appropriate dilution ventilation from the wards and air diffusion into the animal exposure chambers, in particular the cages, to ensure that the animals are exposed of maximum quanta of air borne pathogens from the wards the following hypothesis was developed to support the design of the system.

4.3.1 Dilution (sanitary) ventilation from the wards

Wells [58] demonstrated that the removal of airborne organisms by ventilation follows an exponential die-away curve which is described by the equation:

$$N_t/N_o = e^{-Kt} \quad (4.1)$$

Where: N_o = initial concentration of airborne organisms

N_t = concentration at time t

K = rate of air removal in air changes per hour

The rate of removal can also be expressed as

$$K = Q/V \quad (4.2)$$

Where: Q = flow rate of air from the space in m^3/s

V = volume of space in m^3 (as described in equation above)

This equation applies to space with well mixed air containing aerosols as small as droplet nuclei which have negligible settling velocity.

This equation for a well mixed model leads to a simple description of the equilibrium (steady state) concentration of airborne organisms at all points in the room. If the emission rate of the *M. tuberculosis* bearing droplet nuclei is donated as above by qI in droplet nuclei/hr, the equilibrium concentration N_{eq}, in droplet nuclei/m^3 is given by:

$$N_{eq} = qI/Q \quad (4.3)$$

4.3.2 TB transferred from the wards to animal exposure chambers

It is necessary to ensure that the maximum possible quanta of infectious particles are transferred from the in-patient wards via the *infected exhaust air transfer duct system* to the animal exposure chambers. This is necessary in order to equate as closely as

possible the infectivity of the air in the in-patient wards to that within the exposure chambers (i.e. the quanta ***concentration*** of infectious aerosolised *M. tuberculosis* in the exposure chambers must equate that within the wards).

As the air transferred from the wards is the source of aerosolised *M. tuberculosis* for the exposure chambers, total mass (quanta) of contaminant $\dot{q}$ of aerosolised *M. tuberculosis* in the exposure chamber air will therefore equate to the quanta of aerosolised TB particles within the wards (assuming that an equivalent dilution of approximately 99.99% is achieved by 12 ACH within the wards).

Due to the volume difference between the wards and exposure chambers, the air change rate for each exposure chamber will be higher than that of the wards. The resultant high air change rate (Relevant ACH) for the exposure chambers; however; will have little effect on the quanta of the aerosolised *M. tuberculosis* within each exposure chamber. The quanta of aerosolised *M. tuberculosis* within the chamber $\dot{q}$ should equate to the source, as the air change rate for each exposure chamber is achieved by the ward air carrying the aerosolised *M. tuberculosis*.

According to Sandberg [59] ventilation efficiency is defined as the ratio of the integrals of the concentration of the contaminant in a space:

$$\eta_v = \frac{\int_0^\infty N_{ref}\, dt}{\int_0^\infty N_{ave}\, dt} \qquad (4.4)$$

Where N_{ave} is the average concentration of the room and N_{ref} is the concentration at the reference point (in this case the inlet to the ward extract duct).

Ventilation efficiency at the location where the contaminant is released can also be defined in terms of a measured mean residual lifetime. The ventilation is said to be efficient if a contaminant released at a given location is rapidly removed from the space through the extract system.

Short-term contaminant removal effectiveness ε_{CR}, with constant airflow and incomplete mixing, is described as follows [59]:

$$\varepsilon_{CR} = \frac{\tau_n}{\tau_t} \tag{4.5}$$

Where τ_n is the system average nominal time constant (the ratio of the volume of the space to the rate of change of fresh air to the space) and τ_t is the turnover time or residence time of the contaminant [60]:

$$\tau_t = \frac{M(\infty)}{\dot{q}} \tag{4.6}$$

Hence the contaminant removal effectiveness would be

$$\varepsilon_{CR} = \frac{\frac{V_{room}}{M(\infty)}}{\frac{Q}{\dot{q}}} \tag{4.7}$$

Where $M(\infty)$ is the total mass of contaminant in the room at steady state and $\dot{q}$ is the rate of continuous emission of the contaminant and Q is the volume flow rate to the room. The ventilation effectiveness may be defined as the ratio of:

$$\varepsilon_v = \frac{N_{duct}}{N_{ave}} \tag{4.8}$$

Where N_{duct} is the concentration in the extract duct system and N_{ave} can be some local concentration or an average space concentration, depending on the particular definition. At steady state $\varepsilon_{CR} = \varepsilon_v$ with the appropriate choice of N_{ave}.

Further in a fully mixed situation and where the supply air to the space equals the exhaust air from the space, the concentration in the exhaust N_R will be the same as the average space concentration N_{ave}, (i.e. $N_{duct} = N_{ave}$) thus resulting in $\varepsilon_v = 1$.

In the case of the animal infection chambers, the supply air Q_s to the chambers is the source concentration $N_{source} = \frac{Q_s}{\dot{q}_s}$. The air extracted from the chambers Q_e is at a slightly higher quantity than that supplied. For bio-hazard control reasons, this additional air is required to create the necessary negative pressure relative to the airlocks to and from the chambers and is made up from leakage through the interconnecting doorways between these spaces (Where $Q_e = Q_s + Q_i$). Assuming therefore that $N_{source} > N_{ave}$ as $\frac{Q_e}{\dot{q}_s} > \frac{Q_s}{\dot{q}_s}$ by the amount $\frac{Q_i}{\dot{q}_s}$.

From the above, it can therefore be concluded that the concentration of the animal infection chambers will therefore equate to:

$$N_{ave} = N_{duct} = \frac{Q_e}{\dot{q}_s} = [\frac{Q_s}{\dot{q}_s} + \frac{Q_i}{\dot{q}_s}] = \frac{Q_s + Q_i}{\dot{q}_s} \qquad (4.9)$$

As the exposure chambers are under negative pressure, the exhaust air volume will always slightly exceed that of the supply. The air required to make up the flow difference for this negative pressure differential to be achieved will be the air which will dilute the aerosolised *M. tuberculosis* within the animal exposure chambers. Any dilution of the aerosolised *M. tuberculosis* in the animal exposure chambers will therefore be via air leakage from the adjacent air locks.

The reduction in the concentration of aerosolised *M. tuberculosis* in the animal exposure chambers, with respect to the concentration of aerosolised *M. tuberculosis* in the wards, may therefore result only from the infiltration of air due to leakage into the exposure chamber Q_i.

4.3.3 Air diffusion to animal exposure chambers

To ensure that all guinea pigs are exposed to an equal concentration of aerosolized tubercle bacilli in the exposure chambers, it is necessary to ensure that each cage (housing up to three guinea pigs) is supplied with an equal amount of air under the

same air diffusion characteristics (this is based on the assumption that the transferred quanta of aerosolised *M. tuberculosis* $\dot{q}_s$ from the wards are evenly distributed in the supply air system).

The cages in the two animal exposure chambers are arranged in a matrix of 60 cages each, supported in a wall/floor mounted frame, placed against the rear wall of each exposure chamber.

Each cage is supplied with an equal amount of air via a dedicated plug diffuser (air nozzle). The supply air nozzles form an integral part of the vertical air distribution manifold fixed to the wall behind the cage support frame as shown in Figure 4.3 below.

Figure 4. 3: Exposure room 1 cages; each with a dedicated supply air nozzle

Each "cage supply air nozzle" is adjustable in order to balance its air supply. Thus; when assuming $N_{ave} = N_{duct} = N_{nozzle}$ when achieving an equal distribution of air Q_{nozzle} to each of the cages, the following is noted:

$$N_{duct} = \frac{Q_s}{\dot{q}_s} = \sum_{1}^{n} \frac{Q_{nozzle}}{\dot{q}_{nozzle}} \tag{4.10}$$

The air supplied to each cage via its dedicated nozzle, having the characteristic of a free air jet in a room, is influenced by reverse flows created by the jet entraining the ambient air and is defined as a confined, compact jet [61].

Compact jets are formed by cylindrical tubes with small circular openings being three-dimensional and axi-symmetric at least at some distance from the diffuser opening [61]. The maximum velocity in the cross section of the compact jet is on the axis of the jet stream.

As the supply air temperature is close to the ambient room air temperature, the air jet is considered to be an isothermal jet. As the outlet areas of each nozzle is small compared to the dimensions of the space normal to the jet, the centre line velocity, at the distance from the face of the nozzle to the first half of the cage, will dissipate to below the max allowable air flow within the animal cage. With maximum diffusion within the cage, the desired dispersion of supplied quanta $\dot{q}_s$ will therefore occur within the cage and not beyond the breathing space of the guinea pigs.

Based on the above assumption, the quanta of aerosolised *M. tuberculosis* $\dot{q}_s$ are evenly distributed in the supply air and therefore, all guinea pigs will be exposed to the equally proportioned quanta of aerosolised TB $\dot{q}_s$ from the wards.

4.3.4 Temperature and relative humidity requirements

When considering the design parameters of the environmental control system, attention should be given to the control of temperature, humidity, air velocity and air pressure within both patient and animal rooms.

The in-patient rooms (the internal spaces)

Different patients may require different temperatures depending upon their sex, condition and type of treatment. Whilst the space conditions will be adjustable, the following design parameters shall apply:

- **Ambient conditions:**

  Location: Witbank, Mpumalanga, SA

  Altitude: 1 500m

  Summer temperature (DB): 32°C

	(WB):	20°C
Winter temperature	(DB):	2°C
Daily range:		12°C
Condensing temperature:		45°C

- **Inside conditions:**

Temperature (DB):	22°C ± 1°C
Relative humidity:	50 % ± 10%

The animal exposure chambers

Special attention needs to given to the ventilation system to ensure best temperature, humidity, air velocity and air pressure within the animal rooms.

Guinea pigs are extremely susceptible to respiratory disease and hence a well designed, efficiently operated system will provide the environmental stability requisite for respiratory disease control.

Care must be taken to control velocity and direction of the air to and within the exposure chamber cages and the rooms in which the Chambers are housed, to ensure a "draftless" and even distribution of air. The air supplied to the exposure chambers and hence the animal rooms will be the exhausted air (infected and disinfected), from the patient rooms.

Reported optimal conditions to be maintained within the animal (guinea pig) rooms are as follows:

- **Inside conditions:**

Temperature (DB)	:	22°C ± 1°C
Relative Humidity	:	50 % ± 10%

From a design perspective the above condition can be assured via small individual fan coil units for each animal room.

The temperature/humidity condition of the infected/disinfected air from the patient rooms, entering the exposure chambers, is however dependent on the conditions within the patient areas. This condition is subject to the research protocol for Airconditioning when considering air disinfection by the UVGI fixtures and is adjustable via the Airconditioning (environmental control) system's control management system.

However; as the optimum environmental conditions for guinea pigs seems to match that of humans, it can be reasonably assumed that the conditions within the patient space will be suitable for the guinea pigs housed in the exposure chambers.

4.4 The airborne infectious research apparatus: design solution overview

The AIR facility is attached to the MDR-TB Referral Hospital in Witbank, Mpumalanga. The Hospital is one of 13 dedicated, specialist referral facilities for MDR-TB treatment across the country; currently co-ordinated by the Medical Research Council; to ensure treatment and management of MDR-TB patients under the DOTS-Plus policy of the Department of Health.

Figure 4. 4: Back view of the MDR-TB Referral Hospital, showing the AIR facility in the foreground

Figure 4. 5: Entrance to the AIR facility, at the back of the MDR-TB Referral Hospital

The AIR facility consists of one wing of the MDR-TB Referral Hospital, converted into a self-contained experimental facility consisting of the following:

4.4.1 Clinical unit

The clinical unit, consisting of three two-bed MDR-TB patient wards, are isolated from the rest of the hospital. These patient wards are used to provide human-source infectious aerosols under controlled conditions.

The three two-bed patient wards include a common recreation area, ablution facilities and nursing station. Only patients referred to the adjacent MDR-TB Referral Hospital are selected to take up residence in the clinical unit after eligibility screening and the signing of individual written consent.

The role of these patients is simply to act as the source of infectious aerosols. They agree to stay in the clinical unit for 20 out of 24 hours, with the remaining four hours allowed outside in the fenced-in adjacent yard for recreation and to meet with family and friends (who are not allowed into the AIR facility). Patient exit and entry to the clinical unit is electronically controlled and recorded.

No patients from the adjacent MDR-TB Referral Hospital are allowed into the AIR facility. This requirement ensures against the possible introduction of additional infections which may confound the data.

In line with hospital policy, only patients of the same gender are resident in the clinical unit at any one time. Clinical staff; three registered nurses per day, two per night; is provided for the nursing and care of the patients. Nurses are on duty 24 hours and are supported by the consulting MDR-TB physician who is on 24-hour call.

Figure 4. 6: View of a typical two-bed patient ward

Figure 4. 7: Patient ward showing air extraction vents

The following specification data characterises the wards areas:

Table 4. 1: Ward specifications

Wards	
Dimensions per room	Ward 1: 3000 X 4730 X 2600 (36.89 m^3) Ward 2: 3200 X 4760 X 2600 (39.60 m^3) Ward 3: 2880 X 4750 X 2600 (35.57 m^3)
Temperature control	22°C +/- 2°C
Relative humidity	55% +/-5%
Ventilation	12 - 6 air changes per hour (experiment dependent)
Day/Night cycle	12 hours/12 hours

4.4.2 Animal unit

The integral animal unit, designed to Bio-Safety Level II standards, is completely separate from the clinical unit and consists of two animal exposure chambers with the capacity to house 180 guinea pigs each. Air exhausted from the patient ward suite is transferred under controlled conditions to these exposure chambers.

Special attention was given to the ventilation system to ensure best temperature, humidity, air velocity and air pressure within the animal rooms.

Whilst the purpose is to expose some of the animals to infected air for research purposes, it is equally important to maintain best conditions for those who are to be protected against the infection.

Guinea pigs are bred through a dedicated breeding programme to meet protocol requirements for sex, weight and special-pathogen-free (SPF) status. They are housed under optimal conditions of temperature, humidity and day-night schedules according to South African legislation on the use of experimental animals.

The following specification data characterises the animal exposure rooms:

Table 4. 2: Animal exposure room specifications

Animal exposure rooms	
Dimensions per room	Exposure chamber 1: 2000 X 5750 X 2125 Exposure chamber 2: 2000 X 5750 X 2125
Temperature	22°C +/-4°C
Relative humidity	55% +/-10%
Ventilation	30 ACH
Day/night cycle	12 hours/12 hours
Minimum cage floor size	600 cm^2 per adult
Minimum cage height	180 mm

The animal cages

Whenever it is appropriate, social animals - such as guinea pigs - should be housed in pairs or groups rather than individually, provided that such housing is not in contradiction with the protocol in question. Further, group-housed animals need less space per animal than individually housed animals.

For this reason, three (3) guinea pigs are accommodated per cage. For three fully grown guinea pigs (each having an approx. mass of 950 gm, depending on the sex), a cage having the dimensions of 0.002 m^2 in area and a height of 178 mm. minimum is necessary. A total of 60 cages per exposure chamber will therefore be required for each animal room.

Until the development of the modern plastic rodent caging designs by suppliers of laboratory equipment, there existed great difference of opinion as to whether guinea pig cages should have solid or wire floors.

The advantage of the wire floor design is that bedding is not needed, resulting in substantial savings in labour with respect to cleaning and sterilising processes. The guinea pigs should stay cleaner and drier because water passes through the wire to the pan below and there is no contact with wet or soggy bedding.

The most important feature of the wire flooring is the size of the wire mesh. It must be large enough to let the droppings fall through, but not of a size to catch and break legs. In all cases the wires should be straight, not crinkled, and be welded at their intersections. Wire floors must be designed to offer no lodgement for droppings at corners, along edges or at intersections of reinforcing wires.

Most modern cage designs, being plastic, require bedding. The cages are built with animal observation, protection against airborne infection and ease of watering and feeding in mind.

When considering the research purpose, the wire mesh design for the cage, which would ensure better exposure to the infected air supplied to the exposure chamber, would be more suited.

Guinea pigs are observed daily for specific health indicators and tested monthly for infection through the use of tuberculin skin tests. This procedure involves shaving and depilating a small area on the animal's back where 0.1ml of concentrated PPD is injected into the skin. The resultant hypersensitivity reaction, if any, is read and measured after 24 hours. Animals with clear signs of infection are euthanized for post-mortem investigations, histopathology and microbiology.

Euthanasia is conducted by injection of a lethal dose of sodium pentobarbital which results in cardiac and respiratory arrest and immediate death, according to South Africa legislation and animal ethic standards. At the end of each experiment, all remaining animals are euthanized using the same procedure.

Animal housing and husbandry

When considering the housing and husbandry aspects of care for laboratory animals the following issues required careful consideration:

1. ***Food storage:*** Areas in which diets and diet ingredients are processed or stored should be kept clean and enclosed to prevent entry of pests. The animal food is stored in a walk in cold room maintained at 4°C.

   As the animal rooms do not have natural lighting, a time controlled lighting system is provided to ensure a uniform diurnal light cycle. In the absence of natural light, the animal food is fortified with Vitamin D.

2. ***Water:*** The monitoring of sanitation practices are all in accordance with written protocols.

   The supply water to the guinea pigs is by means of a water bottle and sipper tube. As mortality rates increase significantly when animals are deprived of water, the watering devices are checked regularly.

   Literature research has revealed that adult guinea pigs receiving a green food supplement (usually lettuce), require 50 to 100ml of water per day [62].

3. ***Sanitation:*** Sanitation - the maintenance of conditions conducive to health - involves bedding changes (as appropriate), cleaning and disinfection.

   The frequency and intensity of cleaning and disinfection should depend on what is needed to provide a healthy environment for an animal in accord with its behaviour and physiologic conditions.

   Attention to detail of thorough cleaning is an integral part of the success of maintaining guinea pigs. Obnoxious odours, accumulation of mineral scale from the high concentration of mineral salts in guinea pig urine and the spread of infectious diseases between animals and cages are minimized with the proper sanitation of cages and racks.

As pathogenic organisms are being researched, the animal cages and accessory equipment from the holding and exposure chambers are autoclaved whenever removed from the animal facility.

Mechanical equipment for cleaning and sanitizing cages and accessory equipment are maintained, cleaned and stored in the specially designed cleaning facility.

Assessing the effectiveness of sanitation is of importance to the research program. The monitoring of sanitation practices are all in accordance with written protocols cover all the sanitary processes; these include visual inspection of the materials, monitoring of the washing water temperatures or microbiological monitoring.

4. ***Waste disposal:*** Conventional, biological and hazardous waste, once sterilised, are regularly removed and safely disposed of. Infectious animal carcasses are incinerated on site. The necessary facilities have been provided for sterilisation and incineration.

4.4.3 Bio-safety level II laboratory

A bio-safety level II (BSLII) laboratory, separate from the clinical unit and animal exposure chamber, provides the necessary mycobacteriological support to the AIR facility. Daily microscopy and culture investigations are conducted on sputum specimens from patients and their strains characterized by molecular techniques, including genetic fingerprinting.

Post-mortem specimens from euthanized guinea pigs are investigated by culture and molecular fingerprinting, which enables the linkage of specific infections in guinea pigs to specific patients. The on-site laboratory is linked to the MRC-TB laboratories in Pretoria, where procedures requiring BSLIII levels are done.

Figure 4. 8: View into the BSLII laboratory

Figure 4. 9: The Class II bio-safety cabinet to ensure laboratory worker protection when performing Bacteriological work

4.4.4 The HVAC installation (including the air transfer system to the animal exposure rooms)

The system supplies conditioned ***all outside air***, to the wards, laboratory and accommodation areas in order to achieve the required internal environmental conditions and pressure control relative to the transfer (exhaust) air system from the wards (infected air to animal exposure rooms) as well as the laboratory and ward ablution unit (necessary for safety and to satisfy the statutory regulations with respect to minimum ventilation requirements).

Figure 4. 10: Different views of the HVAC duct system above wards and animal exposure rooms

The "All Air" system is supplied with chilled water, generated by an "Air Cooled" chilled water generating plant. The plant is sized to provide the required cooling and humidification control of the "All Air" system.

Infected air is exhausted from the wards, via a common transfer (exhaust) air system as described in the foregoing paragraphs and pumped to the exposure chambers housed in the animal rooms. This part of the installation forms the most vital component of the research facility's apparatus (the infected/disinfected air must be supplied equally to both exposure chambers).

Pressure relief (exhaust) systems are provided to all of the animal exposure rooms. The air from these rooms is exhausted through High Efficiency Particulate Air (HEPA) filters, to ensure against the spread of infectious micro-organisms to the adjoining hospital areas.

The installation therefore comprises three important systems. These are discussed in more detail below.

a) The air handling plant and reticulation system

The air handling plant provides conditioned air to the above identified areas via a factory manufactured unit, comprising a filtration unit, cooling/dehumidifying coils and variable speed drive supply air fan, connected to the supply air reticulation system. Terminal reheating will be installed to control supply air temperature to individual spaces. Humidification is also provided.

The air reticulation is via externally insulated sheet metal ducts fitted with all the necessary air control and balancing devices to ensure correct air distribution to the identified areas. The duct reticulation is mounted within the ceiling void of the facility.

The air control devices and modulating volume control dampers for space pressure control are fast acting, positive control devices that are easily accessible for

maintenance purposes and ensure ease of calibration during the constant monitoring process as required by the research protocols.

The air is supplied to the various spaces via diffusers and side wall grilles, all selected to limit the space (room) supply air temperature differential, whilst maintaining air supply at the desired rate to ensure maximum air mixing by induction and to provide adequate air diffusion whilst minimizing stratification.

The laboratory areas, whilst being on the same supply system, are controlled separately to ensure appropriate bio-safety levels.

b) The air transfer system from the inpatient areas to the animal exposure chambers

This system is the critical component for the apparatus, as the infected/disinfected air, drawn from the wards is used to expose the guinea pigs to infectious airborne microbes. The mechanical line diagram of the HVAC system is provided in Appendix D.

Air is drawn from the wards and transferred to the animal rooms. The air ducts are divided so that half of the air drawn will be supplied to each of the exposure chambers housed within the rooms.

The ward air is to be delivered unchanged to one of the exposure chambers (the intended test chamber), while the air going to the other (the control chamber), will be disinfected by being intensely radiated with ultra violet light by an in duct UVGI unit.

In order to ensure cross over tests, a UVGI unit will be fitted to both supply ducts. The control of each will be subject to research protocol.

The air volume flow to the exposure chambers will be controlled by fan speed control to ensure correct air flow to the exposure chambers.

Figure 4. 11: Close-up view of an individual air nozzle, designed to allow uniform air movement across each guinea pig cage

These rooms are ventilated to the outside via a fan powered ducted system, which will be fitted with High Efficiency Particulate Air (HEPA) filters to ensure against the spread of infectious micro orgasms. The fan is speed controlled to maintain the desired negative pressure within the animal rooms and to ensure the correct air change rate within the rooms.

In order to comply with the statutory requirements and to ensure correct pressure balance within the inpatient areas; however; the ward ablution facilities are ventilated separately to the outside.

c) The chilled water generating plant

The chilled water generating plant is required to produce the chilled water necessary to achieve cooling and dehumidifying of the filtered ventilation air to be supplied to the wards, laboratory and accommodation unit as well as the animal rooms.

d) The electrical power and electronic control systems

The Motor Control Centre (MCC), housed within the air conditioning plant room, will incorporate all wiring, switchgear for the power and operational control of the HVAC equipment.

The controls shall be of the electronic type and shall incorporate modern, state-of-the-art technology. All control devices shall be connected so as to perform the required function and operate in the required sequence.

The controls shall be Direct Digital Control (DDC), which will provide closed loop control for all HVAC and timed control for identified equipment (UVGI fixtures etc.). Each of the digital controllers shall be a microprocessor-based, multi-tasking, real time digital processor.

Each of the DDC controllers, which will be housed in the MCC, will operate as standalone controllers, capable of performing its specified control responsibility independently of other controllers in its specific network.

The proposed Building Management System (BMS) shall be a fully integrated control system, which will be in line with industry standard protocols such as BACnet, LonMark/LonTalk for process control or integrator interfaces between cooperating manufacturers systems.

The system architecture shall consist of products of a manufacturer regularly engaged in the production of Building Management Systems and shall be the manufacturers latest standard of design. The BMS workstation shall consist of a local personal computer for command entry, information management, network alarm management and database management functions.

All real-time control functions of the various systems; including scheduling for equipment operation, history collection and alarming; shall reside in the BMS controllers to facilitate greater fault tolerance and reliability. It is also proposed that a text interface shall be provided that will allow access to the BMS data via the Internet. This interface shall use web-based pages to send and receive data from the AIR facility's BMS to a web browser. This will allow access; by all concerned with the research work; to the various operating parameters of the electrical and mechanical installations.

e) The HVAC operation and control narrative

Whilst it is recommended that the 3 patient rooms have individual temperature control; as different patients may require different temperatures depending on age, sex, condition and type of treatment; cognizance must be taken of the purpose of the system as required.

As the conditioned air supply (all outside air) to the patient areas is to be exhausted to the exposure chambers, as infected/disinfected air, a variable air volume system; for temperature control; will not be convenient as it would complicate the goals of the various research work envisaged.

It is therefore proposed that the temperature and humidity control in the patient rooms be controlled by automatic control valve modulation at the air handling unit's cooling coils to ensure desired average temperature/humidity within the occupied spaces. Individual space temperature is satisfied by air terminal (diffuser) reheat elements.

The volume adjustment of conditioned supply air and the exhaust air to each of the exposure chambers will also be possible. The supply and exhaust fans will be fitted with variable speed drive control to ensure supply and exhaust air volume control via set point adjustment of the DDC control system.

The intention for the above volume control to and from the inpatient areas is as follows:

- To ensure pressure differentials between identified spaces within the inpatient ward (Ward C).
- To alter the ventilation rate to the wards to accommodate different research protocols for the room conditions, as the need may arise.

- To maintain the critical balance between supply air to the inpatient ward and the exposure chamber in the animal rooms.

- To maintain adequate air flow to the exposure chambers as well as to insure the required air change rate within the animal rooms.

The Relative Humidity (RH) will not be adjustable per individual space. The overall average RH within the patient areas will; however; be adjustable. This is also to satisfy different research protocols for room conditions as needed.

As confirmed above, the control of the UVGI fixtures within the ward and the sequence control of the duct mounted UVGI units will be via the BMS/DDC control system. All control functions and operational set points of the HVAC system will be accessible and controlled via the BMS PC which can be installed in the HVAC plant room or in the accommodation unit of the facility.

4.4.5 The apparatus control systems

Flexible, state-of-the-art direct digital control systems for ventilation, heating, cooling and humidity control enable simulation of different environmental conditions that may affect transmission.

Electronic, web-based systems for monitoring of the clinical unit and animal exposure chambers allow ongoing recording of engineering components (e.g. pressure differentials, temperature, humidity); automatic detection of engineering abnormalities and electronic recording of patient exit and entry into the clinical unit.

Variable access levels of authority for remote control of the clinical unit and animal exposure chambers enable instantaneous intervention if deviations from pre-set engineering parameters are detected.

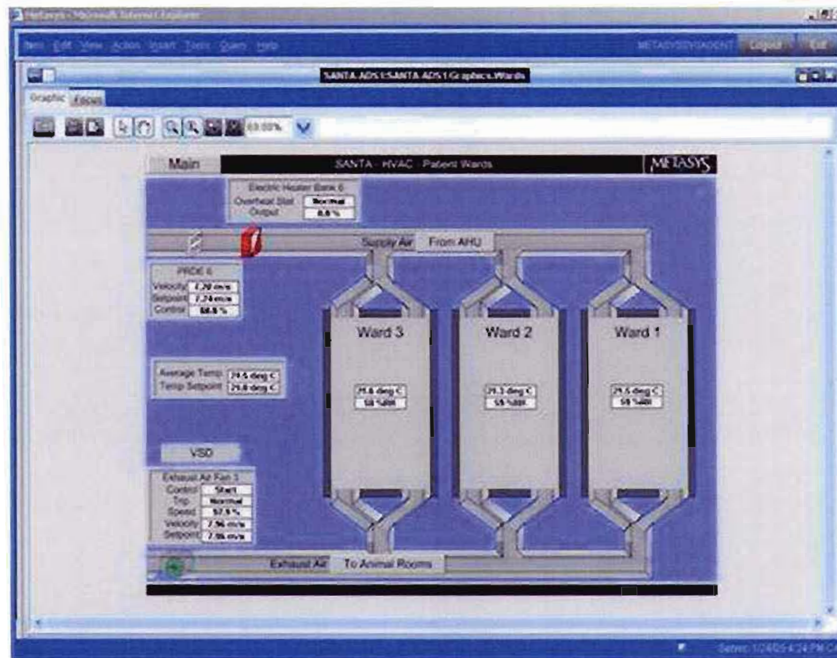


Figure 4. 12: The Building Management System front-end screen showing operational data points of the patient wards

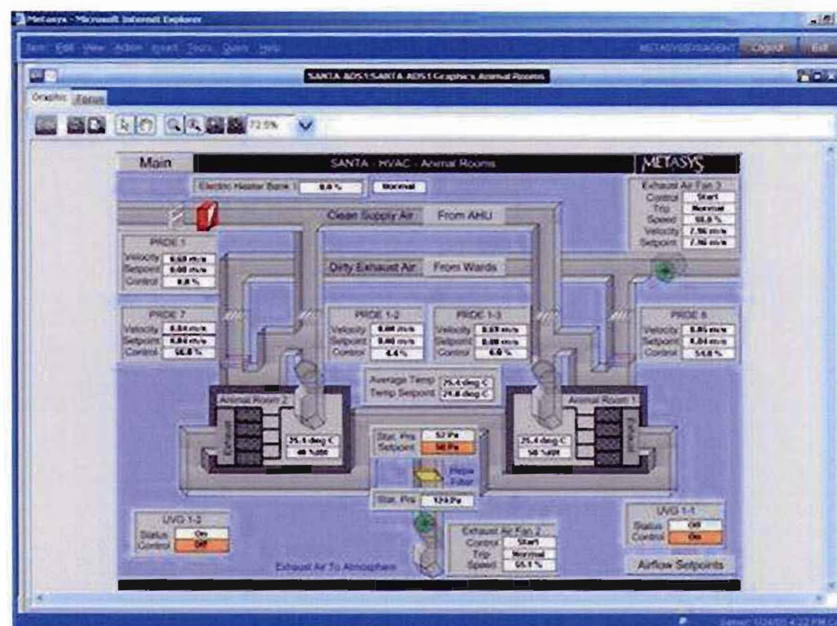


Figure 4. 13: The Building Management System front-end screen showing operational data points of the transfer duct system from the wards to the animal rooms

Electronic controls provide the flexibility to switch filters, fans and/or environmental interventions on and off according to specific research requirements and without the knowledge of either patients or researchers. This allows for comparative assessment of interventions in a blinded fashion (i.e. the guinea pig rooms can be used in a manner that cancels out any potential confounding, such as a particularly infectious patient in one ward who may skew the results).

For example, in studying the effect of an environmental intervention such as UVGI on MDR-TB transmission, one of the animal rooms could be exposed to air from the clinical unit while the intervention is in place. The other room then serves as a control, exposed to air from the clinical unit while the intervention is not in place. Exposure can be alternated between rooms every day to control for variations in infectiousness of individual patients. At any point in time, one animal room would only be exposed to air when the UVGI is switched off and the other room only when the UVGI is switched on.

The difference in the two rooms between those guinea pigs infected and those not would then represent the effect of UVGI on transmission, independent of the absolute infectiousness of the patients in the clinical unit at the time.

4.5 Ethical issues

4.5.1 Patients

Patients diagnosed with MDR-TB in the Mpumalanga Province of South Africa are referred to the MDR-TB Referral Hospital for treatment. All MDR-TB patients in South Africa are treated with a standardized regiment of second-line anti-tuberculosis drugs under the so-called DOTS-Plus policy of the Department of Health. Patients remain in hospital until they have completed the intensive phase of MDR-TB treatment (four months) or preferably until their sputum become culture-negative, at which point they are discharged to continue the rest of their treatment (12 – 18 months) on an outpatient basis.

All newly referred MDR-TB patients are assessed for eligibility into the AIR facility and approached by the hospital research coordinator for possible participation. They are provided with written informed consent documents in their home language outlining the research and their anticipated involvement. The research coordinator also verbally reviews the documentation with patients and gives them enough opportunity to ask questions.

Interpreters are available to obtain consent if needed. If a patient agrees to participate s/he signs or legally marks the informed consent document and takes residence in the clinical unit of the AIR facility. Patients retain the right to change their mind and quit the research at any time and return to the regular MDR-TB ward, with no adverse consequences to their care.

AIR facility patients receive the same standard of MDR-TB care as in the adjacent hospital according to South African MDR-TB treatment policies. No incentives are given to patients to stay in the clinical unit; rather, the wards and communal area have been equipped with television sets and a video machine, various games and reading material. Patients also have access to unlimited refreshments during their stay in the clinical unit, additional to routine meals provided by the hospital.

All patients being treated for MDR-TB under routine DOTS-Plus policies in South Africa are required to submit monthly sputum specimens for laboratory evaluation. AIR facility patients are requested to provide daily sputum specimens during their stay in the clinical unit in order to track their response to treatment relative to their infectiousness to the guinea pigs. The overall duration of stay in the clinical unit depends on the type of experiment being conducted and can range from a few days to several weeks.

Clinical care of patients is provided through standard operating procedures which form part of all study protocols. All AIR facility study protocols are approved by Human Ethics Committees at the MRC, CDC and Harvard University prior to commencement of any research activities.

4.5.2 Guinea pigs

Guinea pigs are specially bred for the AIR facility by the National Health Laboratory Service (NHLS), as they have to be of a specific type (Duncan Hartley strain), female and between 250g and 300g when they take up residence in the AIR Facility. Animals are bred under special conditions to ensure that they are free of other pathogens.

Guinea pigs are kept under conditions complying with the National Code for Animal Use in Research, Education, Diagnosis and Testing of Drugs and Related Substances in South Africa [62]. This code incorporates the principles of laboratory animal science as well as the standards of the US Public Health Service Guidelines for the care and use of laboratory animals. All research meets the ethical standards of both the US and South African Veterinary Medical Associations.

Dedicated and specially trained experimental animal handlers are responsible for day-to-day care of the guinea pigs. They are supervised by an animal technologist with experience in laboratory animal work. All staff members receive training on AIR facility procedures and participate in the MRC health monitoring programme, aimed at preventing TB among laboratory and other high-risk workers.

This programme entails a baseline risk assessment (including history of previous TB, tuberculin skin test status, contact with known TB patients, screening for active TB by chest radiography and sputum bacteriology), a quarterly assessment of respiratory health status and weight and annual chest x-rays and bacteriological examination of sputum specimens. Staff members are also encouraged to undergo HIV counselling and testing. Standard infection control practices and safety procedures are enforced, including the use of personal respiratory protection that prevent the inhalation of infectious particles.

The AIR facility retains as a consultant the services of one of only two laboratory animal veterinarians in South Africa. Health surveillance of animals is conducted daily, while skin testing for infection is done monthly. Animals suspected of being

infected or ill are euthanized by intra-peritoneal injection of a lethal dose of sodium pentobarbital, resulting in immediate death. At the end of individual experiments all remaining animals are euthanized and replaced with new, recently-bred SPF animals.

Animal procedures and care are provided through standard operating procedures which form part of all study protocols. All AIR facility study protocols are approved by Animal Ethics Committees at the MRC, prior to commencement of any research activities.

4.6 Data management

Clinical data for MDR-TB patients are maintained electronically by the hospital as part of the national DOTS-Plus initiative coordinated by the MRC. All data pertaining to treatment of MDR-TB patients under Department of Health policy in South Africa are contained in confidential patient files and entered into an electronic MDR-TB database held at the MDR-TB Referral Centres. Access to the electronic register is limited to accredited staff at the MDR-TB Referral Centre and the international research team.

Data for patients consenting to AIR facility projects are extracted from the electronic DOTS-Plus register and made available through unique patient numbers linked to patients' medical records. Research records of AIR facility patients are maintained in a separate electronic database and kept in strictest confidence according to MRC policies and procedures. Access to AIR facility data is only available to the international research team and reference to study findings is always made by unique study numbers, never by patient identifiers.

All data on the animals is also electronically recorded in a separate research database, maintained at the AIR facility and accessible only to the international research team.

All engineering data is electronically recorded - in real-time - and stored in a separate engineering database. This database is maintained to support the clinical, animal and engineering investigations and is accessible only to the international research team.

4.7 The commissioning (testing and balancing) and operating parameters of the AIR Facility

In the context of inspection and commissioning of the completed AIR facility (apparatus) and all its included systems (where relevant), the following definitions were adopted by the research team:

a) *Inspection* of all systems was by establishing, at completion of each, that the specified and approved provisions for efficient operation had been put in place.

b) *Commissioning* consisted of a series of installation and operational tests by the contractors, suppliers and engineers to establish the advancement of these systems from the state of static completion to working order and that such met with the design specifications (test and balance and where relevant, confirm compliance with health and safety requirements).

Commissioning was therefore defined as the process of testing and balancing and thereby obtaining and documenting evidence that the various installations (equipment) had been provided, installed and that such functioned within the determined limits when operated and were in accordance with the tender/purchase specification.

The objectives of the commissioning process of the AIR facility were therefore:

- To ensure that the mechanical test and balancing of the AIR facility has been satisfactorily undertaken and documented in line with the qualification requirements for the complete apparatus. These tests will include:
    - Test and balancing of the supply and exhaust air systems (including the "infected exhaust air" transfer duct system).
    - Test and balancing of the supply systems to patient and animal areas to ensure comfort and health criteria are satisfied.

- Test and balancing of safety filtration units.

- Test and balancing of the temperature, humidity and pressure controls.

- Test and balancing of the Building Management System, its data logging and communication processes.

- To demonstrate the effectiveness of the ventilation apparatus.

For each system the operational tests - including setting-to-work and regulation (that is testing and adjusting repetitively) to achieve the specified performance, the calibration, setting up and testing of the associated automatic control systems and the recording of the system settings and system performance test results - had been accepted as satisfactory.

4.8 Initial investment cost

The AIR facility initiated by the SA Medical Research Council, CSIR, CDC and Harvard University was developed with funding provided by the United States Agency for International Development (USAID), private sector and the now defunct South African National Tuberculosis Association (SANTA) donations amounting to a total of R 4 500 000-00.

4.9 Conclusions

The vision of the research team in formulating the design process for the facility was to ensure that the operating and maintenance of the equipment met with the scientific rigors of the proposed research work to be undertaken.

The commissioning reports confirmed that every system had been inspected and commissioned in the appropriate sequence and that the operational performance of the installation was in accordance with the design.

This commissioning process will be repeated prior to each of the validation experiments to ensure consistency in results and that the integrity of each new experiment is not compromised due to possible failure or calibration drift of any transducer or component of the apparatus.

The operating conditions within the animal exposure chamber and the standard operating procedures for animal husbandry were approved by the veterinary consultant prior to accommodating any animals. Daily monitoring of conditions in the AIR facility is done electronically (e.g. logging of temperature, humidity and air pressure differentials) and includes the animal infection chamber.

With respect to in-patient accommodation, reports from the hospital confirmed patient comfort and that the layout of the wards was most conducive to providing the necessary level of nursing for patients with MDR-TB (and in most cases; co-infected with HIV).

When considering the functionality of the design of this unique facility, it is has been concluded that it is well suited to facilitate scientific studies to answer fundamental questions about the infectiousness of multi drug *M. tuberculosis*, the role of HIV and the effectiveness of environmental controls to curtail transmission and that it is well suited to provide the scientific blue-prints for design of safer health care facilities and the development of improved building and construction standards internationally.

5

VALIDATION OF THE EXPERIMENTAL FACILITY

Validation is the documented procedure required for obtaining, recording and interpreting the results needed for assurance and confirmation that the various systems installed for functional support, health and safety at the facility will consistently perform and comply with a pre-determined specification.

5 VALIDATION OF THE EXPERIMENTAL FACILITY

5.1 Introduction

The transport apparatus for transferring infectious air between the wards and animal exposure chambers (i.e. the "transfer duct"), including the safety filtration units and electronic control systems were tested for operation and then calibrated with respect to dynamic function during the commissioning (testing and balancing) process, was described in the previous chapter.

In order to be certain that the expected guinea pig infection rates during the calibration experiment of the apparatus accurately reflect the concentration of infectious particles in the exhaust air from the clinical unit, it was necessary to validate the tightness and efficiency (leakage factor) of the transport apparatus and the efficacy (ability to disinfect air) of the in-duct UVGI units to the animal infection chambers were also tested. These in-duct UVGI units allow for one or both animal infection chambers to be protected from contagion from the ward. This feature was provided should any specific experiment require such for control purposes.

Before conducting any biological testing, non-biological aerosols (polystyrene spheres) were generated in the patient ward and sampled with an optical particle counter at various locations along the ventilation path from the wards through to the animal quarters.

Two biological aerosols (endospores of *Bacillus subtilis var. niger* and *Serratia marcescens*) were generated in the patient ward and sampled at various locations between the wards and animal exposure chambers, using six-stage Anderson samplers, for bio-aerosols.

The proportion of inert and infectious material that was recovered in the animal chambers from amounts generated in the wards and ducts is used to determine the efficiency of the transmission system. The interpretation of this data is important for

understanding the transfer dynamics when TB patients generate the infectious material during the various experiments to be undertaken.

5.2 Objectives of the validation of the AIR facility ventilation apparatus

The objectives of the validation experiments on the infection transfer apparatus (the "transfer duct" and "infected exhaust air" systems from the wards to the animal exposure chambers) of the AIR facility were therefore:

- To validate the effectiveness and air tightness (leakage factor) of the air distribution from the wards to the animal infection chambers via the "infected exhaust air" transfer duct system.
- To determine the effective transfer of infectious airborne particles from patient wards to the animal rooms via the "transfer duct" system.
- To determine the efficacy of the in-duct UVGI units installed in the "transfer duct" system.

These studies form the basis for the operational parameters of the apparatus during future experiments. The following conditions and operational functions were set for all experiments:

5.2.1 Patient ward conditions and animal exposure rooms (sample size calculation) during the validation experiments

In the protocol for both of the validation experiments described below, the procedure for validation is described. By varying the ventilation air change rates (12 to 3 ACH) in the wards, the ventilation plant between the wards and the animal exposure rooms will vary between 750 L/s (12 ACH) and 135 L/s (3 ACH).

The patient wards were kept under negative pressure with windows permanently sealed and doors opened to allow for maximum air extraction. The system design

provide for a dedicated air supply and extract system in the ablution areas to ensure that these areas are under neutral pressure relative to corridors, patient rooms and the communal area. These areas are also fitted with automatically closing doors to avoid potential air contamination.

No guinea pigs were used for the validation process. Manual air sampling was undertaken in randomly selected animal cages in the exposure rooms.

5.2.2 Collection of operational data from the facility apparatus

The following trend data was collected electronically and stored in the Building Management System (BMS) during each of the experiments:

- *Environmental conditions of the patient wards*
  (Temperature, humidity and air changes per hour)

- *Environmental conditions of the animal exposure chambers*
  (Temperature, humidity, air changes per hour, HEPA filter pressure differentials and in-duct ultraviolet status)

- *Supply air operating conditions*
  (On- and off-coil conditions, filter pressure differentials, outside air dry bulb temperature (DB) and outside air humidity (% relative humidity))

- *Pressure differentials within the animal rooms and the laboratory*

- *Log of access through all doors within the animal rooms*

- *Equipment alarms*
  (Fan, pump and chiller motor trips)

The above trends were constantly reviewed to identify deviations from set points and/or operational trends. Interrogation of the data was undertaken to provide support for the validation process, with respect to environmental impact or deviation from standards or procedures with regard to facility operation. Alarms were monitored in real time to ensure immediate remedial action.

5.3 Validation experiment 1: Determining the effectiveness of the air transfer system

Hypothesis: *The effectiveness and air tightness (leakage factor) of the Air Transfer System between the wards and the animal exposure rooms is at least 95% effective when conveying airborne concentration.*

Specific Aim: *The aim of this experiment was to demonstrate the effectiveness and air tightness (leakage factor) of the Air Transfer System between the wards and the animal exposure rooms.*

5.3.1 Methodology for validation experiment 1

The effectiveness and air tightness (leakage factor) of the "transfer duct" system from the wards to the animal infection chambers was tested by using uniform Poly-Styrene Latex (PSL) microspheres; sizes 1, 3 and 5 μm.

The specifications and description of the various apparatus (equipment and non-biological aerosols used for validation experiment 1 are described in Appendix D).

Aerosol generation: Validation experiment 1

The patient wards were cleaned by supplying HEPA-filtered outside air for 6 hours prior to the commencement of aerosol generation and sampling.

The monodisperse solid-particles aerosols (uniform Poly-Styrene Latex microspheres (PSL), sizes 1, 3 and 5 μm) were generated in the three patient wards. A six-jet

modified MRE-type Collison nebuliser (refer to appendix D) was used to aerosolise the suspensions of Poly-Styrene spheres.

Nebulising was started at bed level, below the air intakes and allowed to stabilise for 5 minutes. The concentration of solid-particles in the nebulizer suspensions was adjusted to ensure a steady-state concentration in the exhaust air system.

Figure 5. 1: The author Nebulising uniform Poly-Styrene Latex Microspheres (PSL), in one of the patient wards using a six-jet modified MRE-type Collison nebuliser

Sampling: Validation experiment 1

After the nebulising process had begun in the patient wards (at bed level, below the air intakes) and allowed to stabilise for 5 minutes, aerosol counts were measured by using a ARTI Handheld Particle Counter Six (HHPC-6) for each of the particle size distribution (1.0, 3.0, 5.0 μm); in the animal exposure rooms and at each of the transfer duct sampling points.

5.3.2 Results: Validation experiment 1

The results validated the operational parameters of the apparatus. The losses were less than 5% for non-biological substances.

Of the PSL number concentration the results indicated a total decreased <5%, for 0.3 μm <3% and for 5 μm <10%.

5.4 Validation Experiment 2: Determining the transporting capacity of biological aerosols

Hypothesis: *The effective transfer of infectious airborne particles from patient wards to the animal rooms via the "transfer duct" system between the wards and the animal exposure rooms is at least 85% effective in conveying airborne concentration. The in-duct UVGI units installed in the "transfer duct" system have the capability to ensure complete (100%) disinfection of aerosolised bacteria from the wards.*

Specific Aim: *To demonstrate the effective capability of the Air Transfer System to transfer biological aerosols between the wards and the animal exposure rooms and to validate the efficacy of the in-duct UVGI units.*

5.4.1 Protocol (Methodology): Validation experiment 2

The organisms selected for the experiment were Bacillis subtilis var. niger (ATCC #090287) and Serratia marcescens (ATCC #8195).

Bacillus subtilis is a ubiquitous bacterium commonly recovered from water, soil, air and decomposing plant residue. This bacterium produces endospores that are thermo- and desiccation-resistant in the environment.

Serratia marcescens has been isolated from eggs of insectary and are red-pigmented (as opposed to human-isolated *S. marcescens*) and non-spore forming.

The specifications and description of the apparatus (equipment and non-biological aerosols used for validation experiment 2) is described in Appendix D.

5.4.2 Aerosol generation: Validation experiment 2

Aerosols of predominantly single cells of *Bacillis subtilis var. niger* (ATCC #090287) and *Serratia marcescens* (ATCC #8195) were generated in the three patient wards. A

six-yet modified MRE-type Collison nebulizer (BGI Inc., Waltham, Massachusetts) was used to aerosolise the suspensions of bacterial cells.

The patient wards were cleaned by supplying HEPA-filtered outside air for 6 hours prior to the commencement of aerosol generation and sampling.

Nebulising was started at bed level, below the air intakes, and allowed to stabilise for 5 minutes. The concentration of bio-aerosols in the nebulizer were adjusted to ensure a steady-state concentration in the exhaust air to provide a challenge concentration of approximately 1 500 CFU/m^3.

5.4.3 Sampling: Validation experiment 2

After the nebulising process had begun in the patient wards (at bed level, below the air intakes) and allowed to stabilise for 5 minutes, aerosols were collected in triplicate and sized using the Andersen 6-STG samplers loaded with the agar plates at the sampling points along the transfer duct system to and within the animal exposure chambers (the duration for each sample point will be 10 minutes):

1. Upstream of ward exhaust plenum
2. Upstream of ventilator EAF-3
3. Downstream of ventilator EAF-3
4. Branch to Animal Room 1
5. Randomly-selected animal cage in Animal Room 1
6. Branch to Animal Room 2
7. Randomly-selected animal cage in Animal Room 2

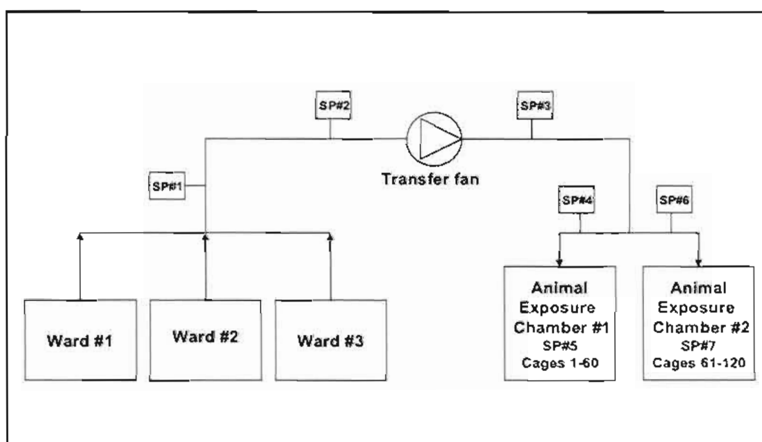


Figure 5. 2: Bioaerosol sampling point positions in transfer duct system and within each animal exposure camber (each position identified as SP)

The loaded 6-STG samplers were placed in the geometric centre of each of the selected animal cages such that the inlet of each will be normal to the air flow and relocated to different cages during the validation process. The sampler in the centre of the room will be at a height of 1500 mm above floor level. The sampling points are shown in Figure 5.2.

After the appropriate sampling time, the air supply to the nebulisers and the sampling vacuum pumps was turned of. The exposure chambers will be allowed to purge the aerosolised bacteria for at least 5 minutes before the samplers are removed from the chamber and aseptically handled.

Figure 5. 3: Air sampling at sampling point 3 along transfer duct, using a 6-STG Anderson sampler

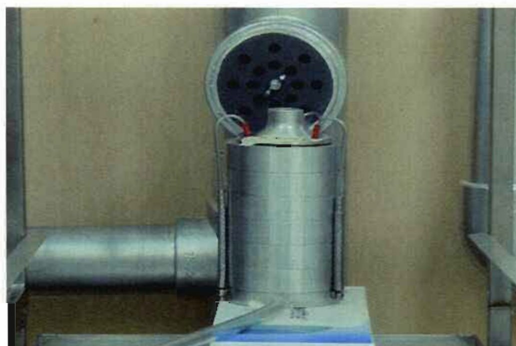


Figure 5. 4: Air sampling at geometric centre of cage position in animal exposure chamber 2, 6-STG Anderson sampler

5.4.4 Enumeration of bacteria: Validation experiment 2

After sampling for approximately 10 minutes and collecting approximately 280 litres of air, the plates will be removed from the sampler, covered, inverted, incubated at 37°C and enumerated after 24 hours (*Bacillus subtilis*, *Serratia marcescens*). Colony forming unit (CFU) counts on all six plates will be combined for analysis.

After 24 hour incubation at a temperature of 37°C, all plates will be enumerated for colony counts. CFU counts on all six plates of each of the 6-Stage Samplers (6- TG's) will be counted, including any number of "multiple hits".

Figure 5. 5: Removing and charging the 6-STG Anderson samplers for air sampling

5.4.5 Results: Validation experiment 2

Biological Aerosols:

- Less than 12% for endospores (Bacillus subtilis) with no significant losses noted across the transfer axial fan.

Evaluation of In-Duct UVGI:

- 100% disinfection efficacy was achieved across the in duct ultraviolet germicidal irradiation units, as no *Serratia marcescens* were detected in the animal room and less than 1 endospore (Bacillus subtilis) per cubic meter of air was detected.

5.5 Conclusions

The vision of the Research Team in formulating the design process for the facility was to ensure that the performance of this apparatus will meet the scientific rigors of the proposed research studies. The achievement of this vision was confirmed by the *Validation Process* of the facility.

6

CALIBRATION OF THE EXPERIMENTAL FACILITY AND STANDARDISATION OF CONTROL PROCEDURES

The objective of calibrating the apparatus is to demonstrate that its functional requirement, namely its ability to quantify the infectiveness of the ward air, is satisfied.

6 CALIBRATION OF THE EXPERIMENTAL FACILITY AND STANDARDISATION OF CONTROL PROCEDURES

6.1 Introduction

Research in the newly-established Airborne Infection Research (AIR) Facility in Witbank, South Africa will continue where the seminal Wells/Riley experiments on transmission dynamics of tuberculosis (TB) [38] [39] [40] [41] left off; focusing on currently unknown aspects of MDR-TB transmission and evaluating the efficacy of engineering controls to minimize the spread of the disease.

These experiments need all to be undertaken within the context of HIV as a significant contributor to the epidemiology of TB in South Africa.

Wells and later Riley [49] emphasised that in undertaking any study into the efficacy of controls of infection, there are a number of requirements which would appear elementary, but are so frequently ignored:

- The first is that, when attempting to obtain a measure of control, the efficiency of the methods employed for interrupting transmission must be thoroughly established.
- The second requirement is that it must be established that the disease is contracted as nearly exclusively as possible within the area under study.
- Thirdly; however carefully methods for the control of airborne infection are devised and applied they will not control infections that are not airborne.

The important decision must always be made at the initiation of a study, whether the method of transmission has been established and is being used to explore the efficiency of the method of control.

The third experiment, namely the biological calibration of the AIR facility, was necessary to demonstrate that infectious particles can be successfully transferred from the clinical unit to the animal exposure chamber and cause infections in the guinea pigs and at standardisation of biological procedures for patient and guinea pig investigations.

A synopsis of relevant literature guiding current decisions on the biological procedures to be employed in the first experiment in the AIR facility is provided in the chapter 1.

6.2 Objectives of the calibration of the facility as an apparatus

The objective of the calibration experiment was to demonstrate that the AIR facility functioned as designed and to quantify the infectiveness of the ward air. No infection control interventions were tested. The patients simply resided on the ward and contributed infectious droplet-nuclei to ward air in the course of their normal activities.

Results from the calibration experiment will be used to refine parameters for subsequent studies, particularly relating to guinea pig numbers, clinical predictors for engineering parameters for optimal functionality of the AIR Facility, MDR-TB patients infectiousness and logistical parameters for guinea pig testing.

Biological variables expected to be influenced in subsequent experiments include the number of guinea pigs infected (which would influence sample size calculations), intervals of patient specimen collection and the relevance of sputum microscopy and culture as predictors of infectiousness.

6.3 The biological calibration of the facility as an apparatus

Hypothesis: *The AIR facility is a highly effective apparatus to quantify the infectiousness of patients and hence determine the infectivity of the air of the wards in which they are housed.*

Specific Aim: *To calibrate the exposure apparatus of the AIR facility in its purpose to effectively transfer infectious airborne particles from patient wards (clinical unit), to the animal exposure chambers, resulting in infection of adequate numbers of guinea pigs (The rate of guinea pig infections observed, will provide evidence that MDR-TB is highly transmissible to people in congregate settings).*

Additional to the above stated specific aim, the following objectives were also identified:

1 To investigate the potential of drug resistant *M. tuberculosis* strains for airborne dispersal, airborne survival and ability to generate infection in guinea pigs exposed to air containing these organisms.

2 To validate and standardise tuberculin testing procedures of the guinea pigs by relating systematically conducted tuberculin skin test results to post-mortem and bacteriological findings.

3 To initiate data collection for eventual correlation of clinical predictors of infectiousness to numbers of guinea pigs infected by specific patients.

4 To characterise the airborne spread of drug resistant *M. tuberculosis* strains circulating in the AIR facility by molecular strain type comparisons between patient and guinea pig isolates, subsequent to exposure to infectious air from the patient wards.

6.3.1 Protocol (Methodology): Calibration experiment

The protocol for the calibration experiment should be read in conjunction with the overall AIR facility protocol developed for ethics clearance at the various institutional review boards. This 3 month study required 28 different patients to occupy the wards.

The ventilation rate from the wards into the exposure chambers was set at a preset rate of air changes per hour to achieve the maximum transfer of aerosols, as described in chapter 4.

This experiment involved the collection of air from the clinical unit (i.e. the wards) where confirmed MDR-TB patients were accommodated; and transporting the ward air, via the "transfer duct", to the guinea pigs housed in the exposure chambers.

The first batch of 360 guinea pigs were moved into the animal exposure chamber. These were subjected to a baseline test prior to the first six patients moving into the clinical unit. The baseline testing confirmed that all animals were clinically healthy. Patients were then recruited to the clinical unit following informed consent.

Operation of the mechanical apparatus: Calibration experiment

During the calibration experiment the mechanical apparatus will be set to maintain the internal conditions for the AIR facility wards and exposure chambers as stated below. The apparatus will also be set to ensure maximum airborne aerosol transfer from the patient wards via the infected exhaust air transfer duct system to the animal exposure chambers. This can only be achieved when the lowest equilibrium concentration of aerosolized tubercle bacilli in the wards is ensured by the ventilation system. The dilution rate in the wards has therefore been set at the operational limit of the apparatus, which will ensure a constant air change rate of 12 air changes per hour (ACH).

The exhaust air flow rate of 420 litres per second from the wards, supplying 210 litres per second to each of the animal exposure chambers (3.3 litres per second per cage supply air nozzle), is maintained by the infected exhaust air transfer system. As this air is the source of aerosolized tubercle bacilli for the exposure chamber, the concentration of aerosolized organisms in the exposure chamber air will therefore equate to the number of aerosolized organisms from source within the wards (assuming that an equivalent dilution of approximately 99.99% is achieved by 12 ACH within the wards).

Due to the volume difference between the wards and exposure chambers the air change rate for each exposure chamber will be higher than that of the wards. The resultant high air change rate (relevant ACH) for the exposure chambers will; however; have little effect on the concentration of aerosolized tubercle bacilli within each exposure chamber. The concentration of aerosolized organisms within each chamber should equate to the source, as the air change rate for each exposure chamber is achieved by the ward air carrying the aerosolized organisms plus air changes (actual ACH) caused by air from leakages into the exposure chambers due to the negative pressure differential relative to the air locks serving each chamber.

As the exposure chambers are under negative pressure the exhaust air volume will always slightly exceed that of the supply. The air required to make up the flow difference for this negative pressure differential to be achieved will be the air which will dilute the aerosolized bacilli in the exposure chamber. Therefore; any dilution of the aerosolized organisms in the animal exposure chambers will be via air leakage from the adjacent air locks.

In order to ensure that guinea pigs are exposed to the highest concentration of aerosolized tubercle bacilli in the exposure chambers it will be necessary to ensure that the airlock doors to and from the exposure chambers are operated only when necessary.

Collection of operational data from the facility apparatus: Calibration experiment

The following trend data will be collected electronically and stored within the Building Management System (BMS). Data will be electronically backed-up weekly:

- Environmental conditions of the patient wards
  (Temperature, humidity and air changes per hour)

- Environmental conditions of the animal exposure chambers
  (Temperature, humidity, air changes per hour, HEPA filters differentials and in-duct ultraviolet status)

- Supply air operating conditions
  (On- and off-coil conditions, filter pressure differentials, outside air dry bulb temperature (DB) and outside air humidity (% RH))

- Pressure differentials within the animal rooms and the laboratory

- Log of access through all doors within the animal rooms

- Equipment alarms
  (Fan motor trips, pump trips and chiller trips)

The above trends will be reviewed weekly to identify deviations from set points and/or operational trends. Interrogation of the data will be undertaken to provide support for the clinical investigations with respect to environmental impact or deviation from standards or procedures with regard to facility operation.

Alarms will be monitored in real time to ensure immediate remedial action.

Ward conditions: Calibration experiment

The patient wards and communal area will be kept under negative pressure and constant temperature of 22 ± 1°C, with windows permanently sealed and doors opened to allow for maximum air extraction.

A dedicated air supply and extract system has been installed in the ablution areas to ensure that these areas are under neutral pressure relative to corridors, patient rooms and the communal area. They have also been fitted with automatically closing doors to avoid potential air contamination.

Patient intake: Calibration experiment

Patients eligible for enrolment were those with *in vitro* confirmed MDR-TB, referred to the MDR-TB Referral Centre attached to the AIR Facility. Exclusion criteria for enrolment were:

- Age less than 18 years;
- Severe debilitation that would prevent regular sputum expectoration;
- MDR-TB treatment already initiated elsewhere; and
- Informed patient consent for participation in research not having been obtained.

For logistical and ethical reasons, only patients of the same gender were housed in the AIR facility at any particular time. A maximum of six patients were housed in the three patient wards at any time.

The first experiment depended on populating of the AIR facility with highly infectious patients. Patient selection was therefore based on the clinical characteristics that are likely to differentiate highly infectious patients (i.e. smear positivity, the presence of cavitary disease on chest radiography and subjective assessment of cough frequency).

On the day of referral, patients underwent the routine DOTS-Plus procedures currently employed by the MDR-TB centre. These include the following:

- Demographic data (name, age, sex, address).
- MDR-TB history (date of initial diagnosis, patient category, site of MDR-TB).
- Medical history (chronic conditions, current medications, allergies, psychiatric history).
- Physical examination (vital signs, heart rate, blood pressure, respiratory rate, height and weight, evaluation of skin, head, neck, oropharynx, cardiovascular system, pulmonary system, abdominal organs, extremities, nervous system).

- Laboratory tests (electrolytes, urea and creatinine, full blood count, urine protein, liver function, sputum microscopy and culture).
- Baseline chest radiograph.
- Baseline audiometry.
- HIV counselling and testing.

Patient information was recorded in the standardised DOTS-Plus forms according to DOTS-Plus policy in South Africa [63].

Cough frequency were documented using a subjective assessment, i.e. patients were asked whether they are coughing to estimate their cough frequency and the nature of coughs.

Patients suspected to be highly infectious (with at least two of the three clinical characteristics, i.e. >= 2+ score on microscopy, cavitary disease on chest x-ray, frequent cough) were approached for possible enrolment. Those who agreed to participate indicated so by signature on a written informed consent form. They were then transferred to the AIR facility where standardised MDR-TB treatment according to the DOTS-Plus programme in South Africa [63] commenced as soon as baseline sputum specimens had been collected.

Patients were not confined to their rooms but were encouraged to spend as much time as possible within the AIR facility in order to optimise the potential for generating infectious aerosols. They were however, allowed to exit the AIR facility on request, for a total period not exceeding four hours per day. Exit and entrance from the Air facility was electronically recorded by the access control system.

During their stay in the AIR facility patients were monitored and followed up according to treatment policy in South Africa. Study-specific information (e.g. cough frequency, daily smear and culture results) was recorded on separate data sheets and kept with the patient treatment case record forms.

Experimental animal procedures: Calibration experiment

- ***Animal husbandry:*** The animal exposure chambers were decontaminated prior to animal accommodation following the standards of procedures developed for the experiment.

  Female, out-bred Dunkin Hartley guinea pigs (250g - 300g) were randomly assigned to cages using a computer-generated randomisation list. Cages in the two animal exposure chambers were coded from left to right and from top to bottom as outlined in Figure 6.1, resulting in a unique identification number for each cage and each animal.

Animal room 1 (cages 1 - 60)

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60

Corridor

Animal room 2 (cages 61 - 120)

61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	88	90
91	92	93	94	95	96	97	98	99	100
101	102	103	104	105	106	107	108	109	110
111	112	113	114	115	116	117	118	119	120

Figure 6. 1: Coding of supply air nozzles and guinea pig cages

Guinea pigs were ear-punched following the standards of procedures developed for the experiment. Each animal was provided an animal identification card inserted within a stainless steel card holder, attached to each cage.

Sterile food supplemented with irradiated hay and sterile water supplemented with water-soluble ascorbic acid was provided *ad libitum*. Standards of procedures for food and water preparation were developed for the experiment. Waste collection trays, water bottles and food trays were cleaned three times per week. Walls and floors of the animal rooms were cleaned once a week. Animals were allowed to acclimatise for a week prior to commencement of the first experiment.

- ***Clinical monitoring:*** Mass measurements were taken at weekly intervals and general health surveillance of guinea pigs conducted three times a week. Clinical observations and mass measurements were recorded in a standardised animal health surveillance data sheet.

- ***Tuberculin skin testing:*** Standard operating procedures for tuberculin testing, reading procedures and processing of guinea pigs were developed for the experiment.

The objective was to allow the 360 animals to be exposed to infectious patient sources for at least a 12 week period. Over this period, four tuberculin skin tests were conducted at four-week intervals in three cohorts of 120 guinea pigs each, starting with a baseline (week 0) skin test prior to exposure. This approach allowed for detection of infected animals before they could develop overt disease, thereby reducing the risk of cross-infection to other guinea pigs in the facility.

A systematic approach was followed to select guinea pigs for testing at the indicated intervals. Cages were randomly selected using a computer-generated list and all guinea pigs within the selected cages were tested using a

standard dose of 100TU/0.1ml protein purified derivate (PPD) (Mycos Laboratories, USA). PPD was prepared immediately prior to testing and administered via the intradermal route on a depilated area of the back of selected guinea pigs.

Reading of skin test indurations (Figure 6.2) was done in a blinded, duplicate manner using digital callipers. Two independent readings were taken at right angles to each other (longitudinal and transversal) at 24 hours post-administration and the results recorded separately in millimetres. The final induration size was calculated as the average from these four readings.

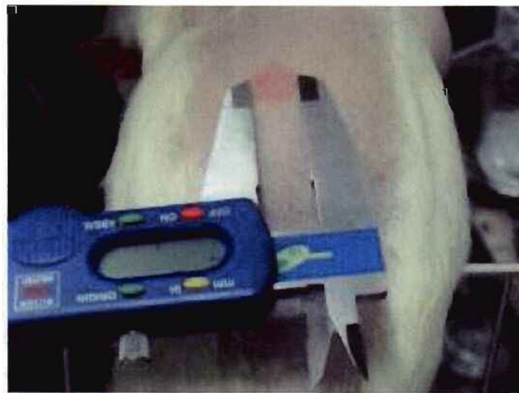


Figure 6. 2: Reading of skin test indurations by digital calliper

Digital photographs (Figure 6.3) were taken of all skin test indurations by the animal technologist using a standardised approach. These were filed with the health surveillance records to facilitate decision-making regarding euthanasia.

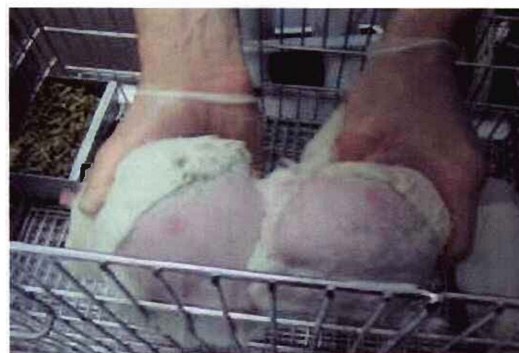


Figure 6. 3: Skin test indurations of an infected guinea pig

Animals with necrotising lesions, as well as those with skin reactions >=10mm and one or more of the following clinical criteria were to be removed and euthanized following final assessment by the veterinary consultant.

At the end of week 14 all remaining animals were monitored for pathology using the above clinical criteria for a further four weeks. At the end of the four week surveillance period a random sample of 25% were selected for bacteriological culture and another 25% for histopathology.

6.4 Results: Calibration experiment

The tuberculin skin test results are provided in Appendix C. Unexposed pathogen-free laboratory guinea pigs repeatedly tested with 100TU of tuberculin PPD showed no reactions as they are unlikely to harbour cross-reacting environmental mycobacteria. From 9 May 2005 through 27 July 2005; however; 82% of the 360 exposed guinea pigs developed PPD reactions equal or greater than 2 mm. Infections occurred throughout the course of the experiment, presumably from multiple infectious patients. This is shown in Figure 6.4.

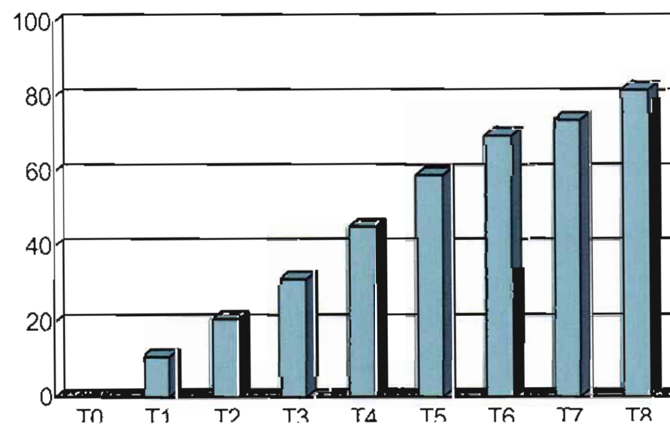


Figure 6. 4: Guinea pig skin test results over 4 months

In Figure 6.4 the Y-axis represents % of 360 guinea pigs infected over a four-month period and the X-axis the baseline (T0) and subsequent testing (T1-8) over four months of exposure and follow-up.

The distributions of skin test reaction sizes were normal, indicating no confounding infection with environmental mycobacteria (Figure 6.5).

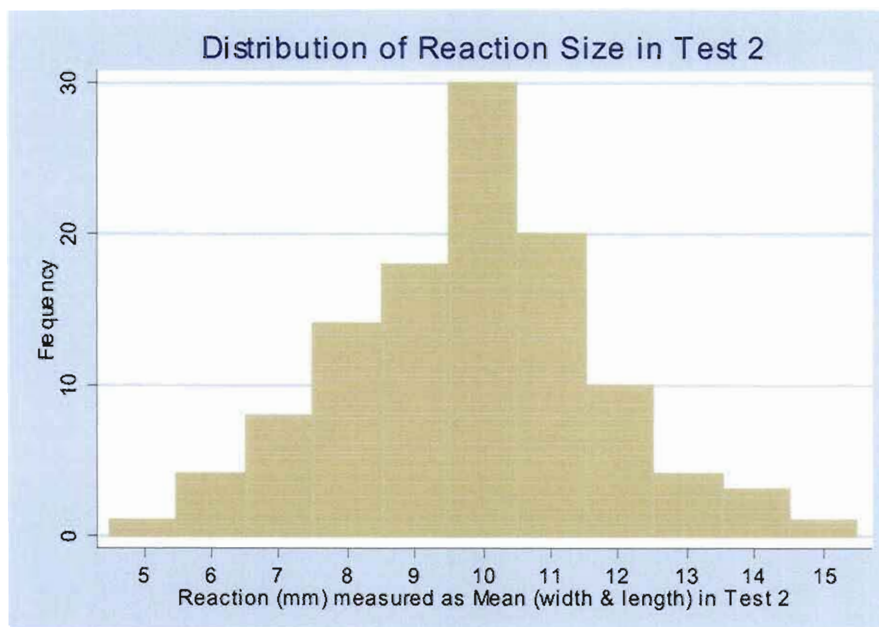


Figure 6. 5: Size distribution of skin test results obtained one month after the start of exposure

Figure 6.5 demonstrates the expected near normal distribution, confirming that immunologic response was not confounded by cross-reacting environmental mycobacterial infections.

Analysis of guinea pig infections indicated no differences in the infection rate between the two exposure chambers. Within each chamber, an analysis by cage location of infected animals demonstrated no clusters of infected animals, indicating random infection from the experimental ward and no cross-infection between animals as shown in Figure 6.5.

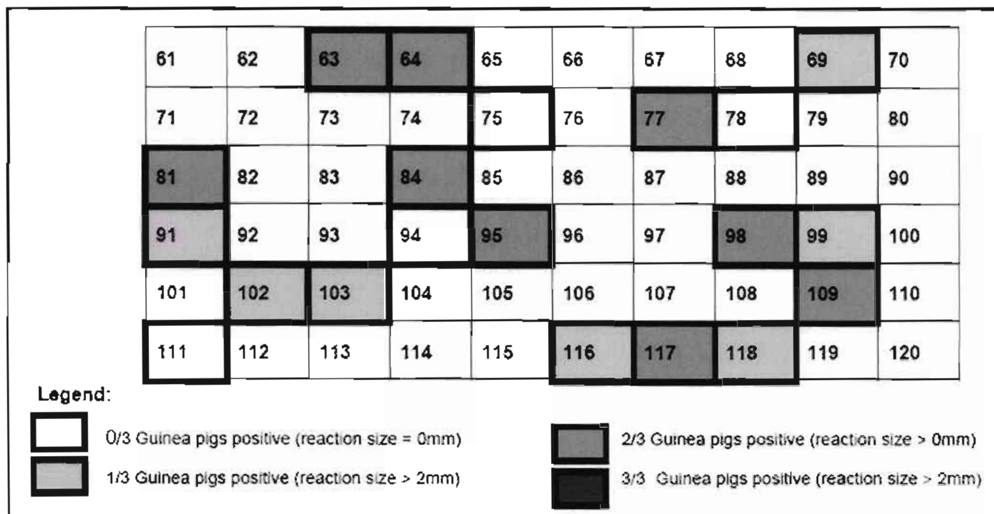


Figure 6. 6: The representative distribution of guinea pig infections, (month one, by cage location in exposure chamber 2 (cages 61 – 120, as if facing the rack of cages in Figure 6.1)

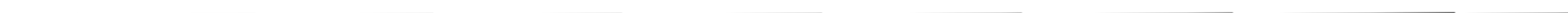
Guinea pigs are not infectious for each other until very late in the disease [64]. Random infection of guinea pigs was also observed by Riley and co-workers [20]. Autopsies and cultures of organs of infected guinea pigs are underway that will permit linkages to specific patient sources.

6.5 Conclusions: Calibration experiment

The AIR facility has been shown to be a highly effective way to quantify the infectiousness of TB patients. Guinea pig autopsies and bacteriology with genotyping will permit an in-depth analysis of clinical and laboratory factors associated with transmission.

The high rates of guinea pig infections observed suggest that drug resistant *M. tuberculosis* may be highly transmissible to people in congregate settings, especially immune-compromised persons. In future studies it will be possible to rapidly assess the efficacy of conventional and novel infection control interventions and health facility designs appropriate for developing countries.

7



CLOSURE

7 CLOSURE

7.1 Summary

It is not possible to culture *M.tuberculosis* from air unless organisms are artificially aerosolized from pure culture [18]. Unlike most respiratory infections; however; human sources of infectious *M. tuberculosis* aerosols are readily available due to the chronicity of this disease and its persistence in many parts of the world, in particular South Africa.

This study covered the development, validation and calibration of the unique Airborne Infection Research (AIR) facility (apparatus) that utilises a biological model to sample airborne *M.tuberculosis* by transporting infectious air from patient wards to animal exposure chambers housing guinea pigs.

This capability, hitherto a universal limitation due to the unique characteristics of the tubercle bacilli, will now allow a collaboration of researchers from around the world to undertake scientific studies to answer fundamental questions about the infectiousness of drug resistant *M. tuberculosis* and the efficacy of various engineering interventions to minimise the spread of airborne disease. These experiments will provide the scientific blue-prints for design of safer health care facilities and the development of improved building and construction standards.

There is an urgent need not only to test conventional approaches to protecting workers, but to develop and then test new, potentially more effective technologies, when they become available.

Clinical field trials of the available interventions are difficult or impossible because of the unpredictable occurrence of most respiratory infections of interest and because of numerous confounding factors such as variable environmental conditions, variable human source strength, variable host susceptibility and variable microbial virulence.

The AIR facility is a collaborative research project between the Medical Research Council, Council for Scientific and Industrial Research (Northwest University), Centers for Disease Control, Atlanta, USA; and Harvard University, Boston, USA; made possible with initial funding provided by the US Agency for International Development (USAID) and private sector donors which included the South African National Tuberculosis Association (SANTA).

7.2 Future work to be undertaken

A project to be funded by Department of Health and Human Services, the Centers for Disease Control and Prevention (CDC) and the National Institute for Occupational Safety and Health (NIOSH) (in the USA) is to be undertaken at the AIR facility and will be titled: *The testing interventions to human-generated occupational airborne infections.*

These experiments are designed to answer fundamental questions regarding transmission and infection control under conditions found in resource-limited countries. The experiments to be undertaken are:

a) **Measuring the efficacy of surgical face masks worn by potentially infectious persons to reduce the generation of infectious aerosols**

Hypothesis: *Surgical masks on the infectious individuals with airborne infections will reduce transmission by at least 50%.*

Specific Aim: *To determine if surgical masks on individuals with airborne infections are at least 50% effective as a means of infection control.*

Surgical face masks worn by potentially infectious persons to reduce the generation of infectious aerosols is an officially recommended method of source control that can be applied quickly in response to an outbreak situation to reduce person-to-person transmission [6]. However; the utility of this simple intervention has never been rigorously tested against human generated aerosols due to the lack of appropriate testing methodology. There is no

substitute for testing persons with airborne infection while wearing masks under real life conditions. This intervention can be readily tested in a controlled experiment in the AIR facility. The results will have important implications for protecting workers and for resource allocation. Many millions of dollars are spent annually on surgical masks for infectious persons around the world in an effort to protect workers and others. This intervention may be effective or it may give workers false confidence. It is vital to know exactly how well this simple intervention works.

b) Measuring the efficacy of portable air disinfection units to reduce the generation of infectious aerosols

Hypothesis: *Portable room air filtration units are at least 75% effective as a means of infection control against human-generated airborne infection.*

- **Sub-hypothesis:** *Flow rate through portable air filtration units is the critical factor in room air disinfection.*

Specific Aim: *To determine if portable air filtration units are at least 75% effective as a means of infection control against human-generated airborne infection.*

Portable air disinfection units can also be applied emergently. The use of portable air cleaners in homes and offices has steadily grown over the last decade and it is now estimated that 1 in 10 American households own some form of device for air cleaning [65]. They are also commonly used in the workplace. They are sold for a range of purposes, from removing smoke to reducing allergens and respiratory infections. Larger commercial devices are used in congregate settings such as hospitals, clinics, nursing homes and homeless shelters. However; the performance of portable air cleaners varies greatly depending on their design and application. An American National Standards Institute (ANSI) testing procedure is designed to evaluate these devices by comparing the decay curves of small, medium and large particulate test contaminants, with and without the device in use [66]. The result is an assigned clean air delivery rate (CADR) for each of 3 test aerosols – smoke, dust and pollen. It is much more difficult to test portable air cleaners against

infectious aerosols and these devices have never been evaluated against human-generated infectious aerosols in a real world setting. The AIR facility was specifically designed for this kind of testing.

Portable air disinfection units utilize a variety of technologies, including HEPA filtration, ultraviolet germicidal irradiation and electrostatic air disinfection. An important metric is the single-pass retention or inactivation rate regardless of the method used [67]. At Harvard they have recently used a variety of aerosolized micro organisms to test a novel multistage electrostatic/filtration air disinfection technology, which had been developed to function for long periods with low maintenance in the Soyuz space capsule. The device demonstrated single-pass inactivation rates approaching 100% for all species, except fungal spores. In this proposal the intention is to test a version of this technology against human-generated aerosol at three different flow rates in well mixed rooms so that a curve of CADR vs. air disinfection efficacy can be drawn. In contrast, because they are inexpensive and already widely available in office settings, it is also proposed to test an office-type portable HEPA filtration room unit to determine its efficacy as a function of its CADR.

c) Determining the efficacy of upper room ultraviolet germicidal irradiation (UVGI) in disinfecting air

Hypothesis: *Germicidal irradiation in recirculation ventilation ducts is at least 75% effective as a means of infection control against human-generated airborne infection.*

- **Sub-hypothesis:** *Mechanical air mixing is an effective way to increase the efficacy of upper room germicidal air disinfection.*
- **Sub-hypothesis:** *High humidity reduces the efficacy of upper room germicidal air disinfection against human-generated airborne infection.*

Specific Aim: *To determine if germicidal irradiation in recirculation ventilation ducts is at least 75% effective as a means of infection control against human-generated airborne infection.*

Air disinfection by upper room ultraviolet germicidal irradiation (UVGI) requires pre-installation as a preventive strategy. One advantage over forced air moving systems is that large volumes of upper room air can be treated at once [50]. Normal room convection currents or mixing fans deliver contaminated air from the breathing space. One early field trial suggested good efficacy against measles transmission in schools [51] and another less rigorous study suggested efficacy against influenza transmission in a hospital [68], but these studies have never been replicated under more controlled conditions against any other human generated infectious aerosols.

Brickner and the Harvard members of the research team [69] conducted a multi-site, placebo controlled trial of UV air disinfection to reduce *M. tuberculosis* transmission in homeless shelters, but low infection rates under placebo conditions and poor retention of homeless subjects led to inconclusive results. Several groups have studied aerosolized test organisms in both small and room-scale exposure chambers and have confirmed high rates of air disinfection [70] [71] [72] [73] [74]. Consistently, room air mixing has been found to improve UV air disinfection while high humidity has been found to diminish it [15] [71] [72] [75] [76] [77]. The aim is to test the efficacy of upper room UVGI against human-generated aerosol and to measure the effects of room air mixing and high humidity under real world conditions.

7.3 Proposed future work

a) Investigate the Wells/Riley equation

Hypothesis: *The Wells/Riley equation implies that the risk does not change over time. The equation should be modified as the risk of infection is in fact not constant over time.*

Specific Aim: *To investigate whether risk is in fact not constant over time, which may well provide argument for modifying the Wells/Riley equation.*

Although some work has been done to investigate the appropriateness of the Wells/Riley equation (Appendix D refers), especially in terms of the actual recorded data from the calibration experiment, there was not sufficient evidence to conclude how appropriate the formula is. Further investigation, and especially further data collection, will be required to be able to finish this investigation.

7.4 Conclusions

As shown in this study, the AIR facility provides the scientific world with the ability to undertake air sampling of human-generated infectious *M. tuberculosis* using guinea pig air sampling. Even after the initiation of effective treatment, the calibration experiment proved that patients remained infectious long enough to generate infectious aerosols against which interventions can be tested.

The contribution of this sampling ability provides the elusive scientific capability to study infectiousness of airborne TB contagion and the efficacy of the much needed engineering interventions to minimise the spread of disease.

The preliminary experiments described in this study indicate that the AIR facility functions as designed and will meet the expectations of such an apparatus in satisfying the rigors of scientific work. Variable host susceptibility as well as variable environmental conditions are eliminated in the experimental apparatus design.

The guinea pig remains a remarkable living air sampler for human-generated *M. tuberculosis* aerosol. It is exquisitely sensitive to the infection, becoming fatally infected by as little as a single inhaled infectious droplet. Unlike culture systems, the guinea pig ignores high ambient concentrations of competing microbes in air.

The high rates of guinea pig infections during the calibration experiment indicate with confidence that the facility can be used for the testing of infection control interventions in a highly efficient manner.

From the conclusions of the investigation of the Wells/Riley equation to determine the spread of airborne infection (Appendix D), using the sample data observed during the Calibration experiment (Appendix C), it was observed that although the risk of infection changed over time, there is a need for more time studies to be conducted on the guinea pigs to get a better idea of how the risk of infection changes over time.

One of the reasons why this risk of infection is of interest is because the Wells/Riley equation uses an exponential survival distribution that implies that the hazard does not change over time. A more detailed study may reveal that the risk is in fact not constant over time, which may well provide argument for challenging the Wells/Riley equation.

The consequential contributions of this study are:

- *The two-animal exposure chamber facility allows experiments to be conducted in a controlled manner, resulting in direct measurements of the efficacy of interventions against the same human source strength of infectious aerosol.*

- *Unlike most clinical field trials, different interventions can now be compared to each other under identical conditions of host susceptibility (guinea pigs), physical environment (the AIR facility) and human infectious source strength (the six patients on the ward).*

- *Moreover, high rates of infection in initial experiments indicate that multiple interventions can be tested sequentially over relatively short time periods compared to most clinical trials.*

- *Variable host susceptibility as well as variable environmental conditions are eliminated in this experimental apparatus design.*

- *The results from a short statistical study to investigate the Wells/Riley equation, has indicated that whilst the equation implies that the risk does not*

change over time, more detailed studies may well reveal that the risk is in fact not constant over time, suggesting that the Wells/Riley equation may need modification.

The consequences of *M. tuberculosis* transmission in congregate settings become distressing when MDR-TB combines with HIV co-infection. Both MDR-TB and HIV are rising in South Africa and many other "resource-limited" countries [93].

The uniqueness of the AIR facility will provide the opportunity to evaluate novel infection control technologies, taking advantage of the latest advances in molecular biology and genetic engineering.

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Appendix A:
THEORETICAL PROCESS OF
DILUTION VENTILATION,
FILTRATION AND
ULTRAVIOLET GERMICIDA IRRADIATION

Appendix A: THEORETICAL PROCESS OF DILUTION VENTILATION, FILTRATION AND ULTRAVIOLET GERMICIDAL IRRADIATION

A1 The theoretical process of dilution ventilation [78] [79]

It is useful to picture how dilution ventilation works within an occupied space. Consider air moving through a duct. It essentially travels a straight path from point A to point B and the volume of air moved, is defined by the physical dimensions of the ductwork.

This type of airflow is called plug-flow or ventilation by displacement. However; air in an occupied space does not move like this. Air comes into the space and mixes with the air already in the room by turbulent diffusion.

The degree of mixing depends upon physical properties of the droplet nuclei, the air velocity, the geometry of the interior of the room with furnishings, and the placement and characteristics of air supply and exhaust vents. In the well-mixed case, after one hour at one ACH, only 63% of the original room air is actually replaced. In a space where the air is "less than well mixed", also called "short-circuited", the amount of air replaced will be less than 63%. In a poorly mixed room, one can conceive of several zones, each one subject to different replacement rates.

In a "well-mixed" room, if there is an initial contaminant concentration N_o, and if no additional contaminant is released, the decline in contaminant concentration due to dilution ventilation is described by the following equation:

$$N_t = N_o e^{-(K)t} \quad \text{(A.1)}$$

Where: k = Q/V

N_o = initial concentration of the gas or vapour

N_t = concentration of the gas or vapour at time t, hrs

K = room air changes per hr

Q = nominal airflow rate, m^3/hr

V = the room volume, m^3

This equation also applies to "well-mixed" air containing aerosols as small as droplet nuclei, which have negligible settling velocity, but it does not apply to any intermittently released gas or aerosol. The CDC guidelines [28] have developed the following table listing the time required for the removal of various percentages of airborne contaminants (this being based on Equation (A.1) and the well-mixed room assumption).

The times provided in Table A.1 assume perfect mixing of the air within the space (i.e. mixing factor = 1). However; perfect mixing usually does not occur, and the mixing factor could be as high as 10 if the air distribution is very poor. The required time is derived by multiplying the appropriate time from the table by the mixing factor that has been determined for the booth or room. The factor and required time should be included in the operating instructions provided by the manufacturer of the booth or enclosure, and these instructions should be followed.

Table A. 1: Air changes per hour (ACH) and time in minutes required for removal efficacy of 90%, 99% and 99,9% of airborne contaminants [28]

ACH	Minutes required for the removal of airborne contaminants		
	90%	99%	99,9%
1	138	276	414
4	69	138	207
3	46	92	138
4	35	69	104
5	28	55	83
6	23	46	69
7	20	39	59
8	17	35	52
9	15	31	46
10	14	28	41
11	13	25	38
12	12	23	35
13	11	21	32
14	10	20	30
15	9	18	28
16	9	17	26
17	8	16	24
18	8	15	23
19	7	15	22
20	7	14	21
25	6	11	17
30	5	9	14
35	4	8	12
40	3	7	10
45	3	6	9
50	3	6	8

This table has been adapted from the formula for the rate of purging airborne contaminants as follows:

$t_1 = \{\ln(N_t + N_0) + (Q \div V)\} \times 60$, with $t1 = 0$, and $N_t + N_0$ - (removal efficiency $\div 100$), and where:

	t_1	= initial timeout
	N_t	= initial concentration of contaminants
	N_0	= final concentration of contaminants
	Q	= airflow rate (cubic feet per hour)
	V	= room volume (cubic feet)
and	k	= Q / V (ACH)

The "well-mixed" room model described by equation (A.1), leads to a simple description of the equilibrium (steady-state) concentration of contaminant at all points in the room. If the emission rate of contaminant, say, *M. tuberculosis* bearing droplet nuclei, is denoted by $\boldsymbol{q}$, the equilibrium concentration N_{eq} is given by:

$$N_{eq} = q/Q \quad \text{(A.2)}$$

Although the well-mixed room assumption leads to simple mathematical descriptions of contaminant concentrations in room air, it happens that most rooms are not well mixed. If conceptualising a room as a "well-mixed" box is the simplest model, the next simplest model that accounts for imperfect air mixing is a two-box or two-zone model. For example, investigators conceptualised a TB patient room as divided into an upper zone and a lower zone (Riley, 1971) [80].

Air within each zone was assumed to be perfectly mixed, but the air exchange between the zones was assumed to be limited. Assume that air is mechanically supplied to and exhausted from the upper zone at rate Q in m^3/hr, that air exchanges between the two zones at rate β in m^3/hr, and that a TB patient located in the lower zone releases infectious droplet nuclei at rate $\boldsymbol{q}$. The equilibrium concentrations of infectious droplet nuclei in the lower zone and upper zones, denoted $N_{L,eq}$ and $N_{u,eq}$ respectively, are given by:

$$N_{L,eq} = q/Q + q/\beta \quad \text{(A.3)}$$

$$N_{u,eq} = q/Q \quad \text{(A.4)}$$

In this model, the lower-zone concentration $N_{L,eq}$ is the exposure intensity of healthcare personnel entering the room. First, note that $N_{L,eq}$ is greater than $N_{u,eq}$ and that the degree of difference depends on the inter-zone airflow rate β. If $\beta \leq Q$, then $N_{L,eq}$ will be at least twice the value of $N_{u,eq}$.

If $\beta >> Q$, then $N_{L,eq} \approx N_{u,eq}$, noting that $N_{u,eq}$ in Equation (4) is the same expression as N_{eq} in equation (A.2). In other words, if the inter-zone airflow rate β is a large value,

the two-zone model essentially becomes the well-mixed room model. However;, in a poorly mixed room, it is more likely that $\beta \leq Q$, in which case the well-mixed room model would significantly underestimate exposure intensity.

Analogous to equation (A.1), there are concentration decay functions for the two-zone model that have the form:

$$N_{L(t)} = \acute{\alpha}_1^{e\lambda 1t} + \acute{\alpha}_2^{e\lambda 2t} \quad (A.5)$$

$$N_{u(t)} = \acute{\alpha}_3^{e\lambda 1t} + \acute{\alpha}_4^{e\lambda 2t} \quad (A.6)$$

The parameters $\acute{\alpha}_{1,}$ $\acute{\alpha}_{2,}$ $\acute{\alpha}_3$ $\acute{\alpha}_4$, $\lambda 1$, $\lambda 2$ in the above equations depend on the values of Q and β, on the volumes of the upper and lower zones, and on the initial concentrations in the upper and lower zones. The fact that the concentration decrease in a two-zone room is not a simple exponential function, as is equation (A.1), can lead to overestimating an effective contaminant removal rate from a decay curve.

While the upper-zone/lower-zone model is a more realistic description of spatial variability than the well-mixed room construct, it also predicts that the contaminant concentration is uniform throughout the lower zone.

However; theory would indicate that contaminant concentrations would be higher close to the point of emission. If many MTB infections occur due to being in close proximity to the source (e.g., a healthcare worker attending a TB patient), the upper-zone/lower-zone model may seriously underestimate exposure intensity and the risk of infection.

To account for imperfect air mixing in the lower zone, the next simplest construct is a three-zone model. The air space around the contaminant source, the TB patient, is the "near-field" zone. As before, the remainder of the room is divided into an upper zone and a lower zone. In this case, the airflow rate between the upper and lower zones is denoted β_2 in m^3/hr, and the airflow rate between the lower zone and the near-field

zone is denoted β_1 in m^3/hr. Given the physical geometries involved, it will usually be true that $\beta_1 << \beta_2$.

If the TB patient releases infectious droplet nuclei at rate *q*, the equilibrium concentrations of infectious droplet nuclei in the near-field, lower and upper zones are, respectively:

$$N_{NF,eq} = q/Q + q/\beta_2 + q/\beta_1 \qquad (A.7)$$

$$N_{L,eq} = q/Q + q/\beta_2 \qquad (A.8)$$

In this model, the near-field concentration, $N_{NF,eq}$, represents a healthcare worker's exposure intensity when in close proximity to a TB patient and the lower-zone concentration, $N_{L,eq}$, is a healthcare worker's exposure intensity elsewhere in the room.

It is noted that $N_{NF,eq}$ is greater than $N_{L,eq}$ and that the degree of difference depends on the inter-zone airflow rate β_1.

if $\beta_1 \leq 0.5\ \beta_2$ and $\beta_2 \leq Q$,

then $N_{NF,eq}$ will be at least twice the value of $N_{L,eq}$.

if $\beta_1 >> \beta_2$,

then $N_{NF,eq} \approx N_{L,eq}$.

The three-zone model then becomes the upper-zone/lower-zone model.

Equations (A.7) and (A.8) for the three-zone model provide a framework for understanding the potential efficacy of controls that increase the *M. tuberculosis* removal rate from the room air. One such control is the cleaning or removal of contaminants from the air via filtration.

A2 The theoretical process of filtration

A2.1 In-room filtration

The CDC [28] guidelines state that while the ability of HEPA filters to remove tubercle bacilli from the air has not been studied, they are likely to be effective since they have been shown to remove spores of the fungus *Aspergillus* which range in size from 1,5 μm to 6 μm, to below measurable levels. However the ability of a HEPA filter to remove particles of a certain size, such as 1,5 μm to 6 μm of *Aspergillus* spores or 1μm to 5μm *M. tuberculosis* bearing droplet nuclei, only addresses the issue of filtration efficiency.

Any properly manufactured HEPA filter should perform according to its stated efficiency and construction rating if installed properly. The more important issue, however; is the ability to *effectively* remove *M. tuberculosis* bearing droplet nuclei from contaminated air *in the room before exposure occurs*, when mounted in an in-room filtration unit.

The effectiveness of free-standing HEPA room air units to exhaust aerosol within a mock hospital room have been preliminary evaluated [81]. These evaluations are significant in that all tests undertaken indicated that the test aerosol concentrations were the highest closest to the release point. This is consistent with what is known of aerosol behaviour and room air mixing.

Expected or not, it is a significant finding because any healthcare activity demands close proximity to the infectious patient.

With HEPA filtration, consider the installation of a ceiling-mounted HEPA filtration unit that moves air through the filter at rate Q_{Filter} in m^3/hr. In effect, Q_{Filter} is an increment to the dilution ventilation rate acting on the upper zone. Prior to installation, the equilibrium concentrations are given by Equations (A.7) and (A.8), but with post-installation, the equilibrium concentrations are:

$$N_{NF,eq} = q/(Q + Q_{Filter}) + q/\beta_2 + q/\beta_1 \qquad (A.9)$$

$$N_{L,eq} = q/(Q + Q_{Filter}) + q/\beta_2 \qquad (A.10)$$

$$N_{u,eq} = q/(Q + Q_{Filter}) \qquad (A.11)$$

Depending on the relative values of Q, Q_{Filter}, β_1 and β_2, the use of the ceiling-mounted HEPA filtration unit might cause only a small decrease in $N_{L,eq}$ relative to equation (A.8) and even a smaller decrease in $N_{NF,eq}$ relative to equation (A.7).

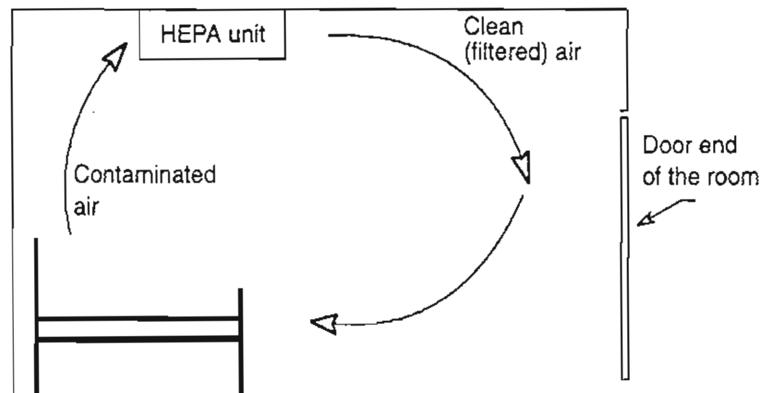

Figure A. 1: Fixed ceiling-mounted recirculation system using a high efficiency particulate air (HEPA) filter

A caveat is that the air motion induced by the HEPA filtration unit might also serve to increase the values of β_1 and β_2 and thereby further decrease the concentrations in the near-field zone and lower zone. Note that if the HEPA filtration unit is mounted on the wall in the lower zone rather than on the ceiling (in the upper zone), Q_{Filter} is an increment to the dilution ventilation rate acting on the lower zone and slightly different equilibrium concentrations are attained:

$$N_{NF,eq} = q/Q + q/(\beta_2 + Q_{Filter}) + q/\beta_1 \qquad (A.12)$$

$$N_{L,eq} = q/Q + q/(\beta_2 + Q_{Filter}) \qquad (A.13)$$

$$N_{u,eq} = q/Q \quad (A.14)$$

Again, depending on the relative values of Q, Q_{Filter}, β_1 and β_2, wall mounting of the HEPA filtration unit might be more effective than ceiling mounting.

A.2.2 Filtration within ductwork

The method of ceiling-mounted filtration units preferred and advocated for use by the CDC are the recirculating stand-alone systems. Air is exhausted from the room into a duct (not forming part of any HVAC duct system), filtered through a HEPA filter in the duct and returned to the room.

As noted in the introduction to this section, HEPA filters can be placed within the HVAC ductwork to remove *M. tuberculosis* bearing droplet nuclei from the space air. However; unless the air exhausted from rooms used to treat patients with suspected infectious TB should be exhausted to the outside in accordance with the statutory requirements, there is no reason to filter this air unless it can re-enter the building ventilation system supply.

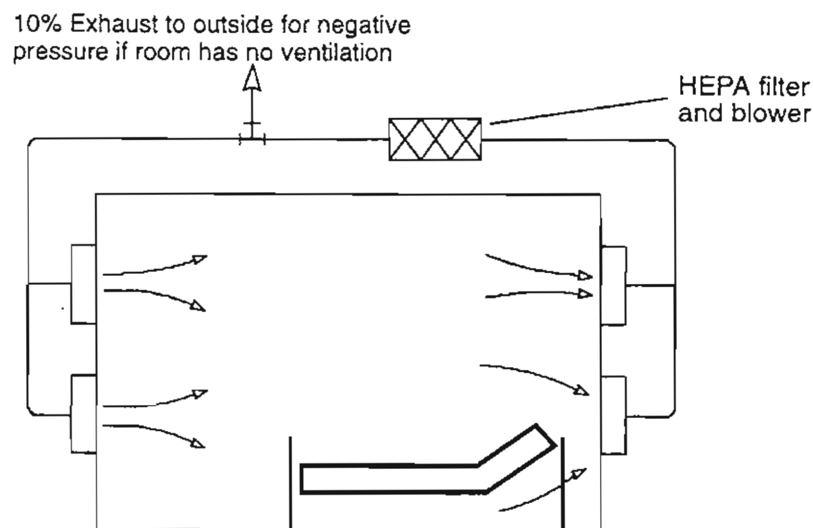

Figure A. 2: Fixed, ducted room-air recirculation system using a high-efficiency particulate air (HEPA) filter inside an air duct

HEPA filtration in the HVAC system cannot lower the airborne concentration of TB droplet nuclei *within* the room from which it is being exhausted.

A3 The theoretical process of ultraviolet germicidal irradiation air disinfection

A3.1 The theoretical model for dilution ventilation with "UVGI air disinfection"

The potential efficacy of UVGI can be understood in an analogous fashion because UVGI, like a ceiling-mounted HEPA filtration unit, increases the removal rate of viable *M. tuberculosis* from the upper zone of the room. The additional removal of *M. tuberculosis* bearing from upper-room air due to UVGI can be expressed in terms of an equivalent dilution ventilation rate Q_{UVGI} in m^3/hr acting on the upper zone. While the equilibrium concentrations prior to UV lamp installation are given in paragraph A1, by equations (A.7) to (A.9), the equilibrium concentrations post-installation are:

$$N_{NF,eq} = q/(Q + Q_{uvgi}) + q/\beta_2 + q/\beta_1 \qquad (A.15)$$

$$N_{L,eq} = q/(Q + Q_{uvgi}) + q/\beta_2 \qquad (A.16)$$

$$N_{U,eq} = q/(Q + Q_{uvgi}) \qquad (A.17)$$

As was the case for a ceiling-mounted HEPA filtration unit, UVGI might effect only a small decrease in $N_{L,eq}$ relative to Equation (A.8) and a smaller decrease in $N_{NF,eq}$ relative to equation (A.7), depending on the relative values of Q, Q_{UVGI}, β_1 and β_2

When considering the use of UVGI, however; it is necessary to appreciate the health effects of UV radiation exposure.

A3.2 Health effects of ultraviolet radiation exposure

Ultraviolet radiation (UV) is a non-ionising radiation and is that portion of the electromagnetic radiation spectrum from 100 to 400 nm wavelengths. For

convenience the UV spectrum has been subdivided into three different wavelength bands: UV-A (long wave lengths, range: 320–400 nm), UV-B (midrange wavelengths, range: 290–320 nm) and UV-C (short wave lengths, range: 100–290 nm) [56].

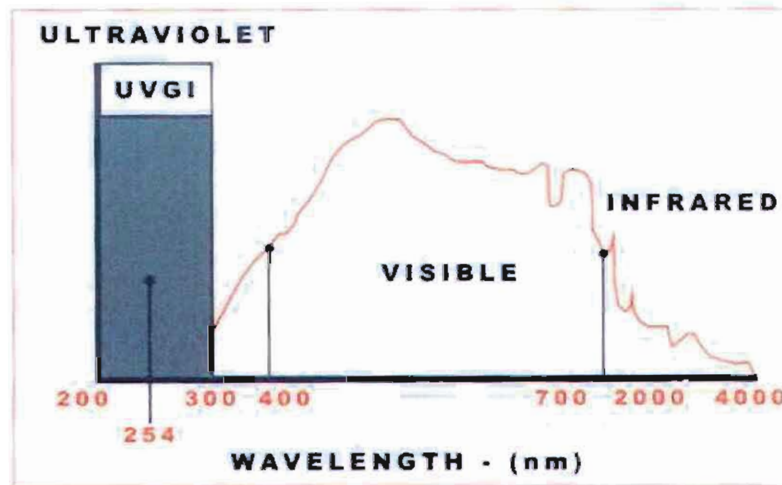

Figure A. 3: Electromagnetic spectrum

UV-C is unlike UV-A and UV-B, as it has extremely low penetrating ability. It is nearly completely absorbed by the outer layer of the skin (stratum corneum), where little or no harm is experienced. Although listed as a potential carcinogen for man, UV-C is unlikely to be carcinogenic or to cause skin or eye irritation (keratoconjunctivitis) if applied correctly within exposure limits as set out by the International Radiation Protection Agency (IRPA) [82] and other international health bodies.

Various biological effects have been established in humans and laboratory animals. The skin, immune system and eyes are target organs for UV. The potential hazard to the skin and eyes varies with wavelength and is indicated by an action spectrum. This spectrum is composed of "reciprocal values of threshold doses for some effect". The action spectrum is used to express the biological efficacy throughout the UV spectrum normalised to the most effective wavelength or bandwidth, for example, 297 nm for the skin and 270-280 nm for the eyes [83].

Ultraviolet radiation exposure could result in acute and chronic effects, such as erythema (diffuse skin redness) and photosensitisation.

- *Erythema* is an inflammatory reaction of the superficial blood vessels involving dilation and increased permeability of the vessels, increased blood flow and cellular exudation (oozing). Experimental human data indicate that there is an area of maximum response around 250 nm, a broad minimum around 280 nm and a second area of maximum response around 297 nm. Erythema follows a dose-dependent latency period of 2–10 hours. Erythema also produces cellular damage and may also produce oedema and blistering.

- *Photosensitivity* involves an abnormal skin reaction to ultraviolet radiation in the presence of certain chemical agents, for example coal tar, colognes, lipsticks, cosmetics, hair preparations, psoralens and sunscreens. Photosensitivity reactions are characterised by eruptions on exposed parts of the body, for example the face, arms and hands. Photosensitivity reactions are either phototoxic or photoallergic. The former is more common than the latter and can be of either an acute or chronic nature. Phototoxicity is a reaction between ultraviolet radiation and a phototoxin and is clinically described as erythema (with or without oedema), followed by hyperpigmentation and shedding of the epidermis. Photoallergy is an acquired immune response, which may include uriticarial or eczematous (chronic dermatitis) lesions. Typical photoallergens include some salicylanilides and antibiotics, hexachorophene, cosmetics and colognes. UV-C and UV-B wavelengths of less than 300 nm are highly absorbed by the corneal tissues of the eye. As the UV wavelengths increase beyond 300 nm, they are increasingly absorbed by the lens of the eye.

- *Photokeratitis and conjunctivitis* are dose-dependent inflammations of the tissues of the cornea and conjunctiva, respectively. The most effective wavelengths are in the 270–280 nm band. Typically, the onset of signs and symptoms follows overexposure by about 6–12 hours although they have been observed in as little as 2 hours and as long as 24 hours. Signs and symptoms

include the following: pain, blepharospasm (twitching, spasmodic contraction), tearing, and congestion of the conjunctiva, photophobia, visual haze and a scratchy feeling in the eyes. In addition, erythema may develop on the eyelids and skin around the eyes. These effects may be incapacitating for up to 2 days. Permanent effects, however; are rare.

Due to the health effects of ultraviolet radiation exposure, the American Conference of Governmental Hygienists (ACGIH) [83], the National Institute of Occupational Safety and Health (NIOSH, 1972) [84] and the International Radiation Protection Association (IRP A, 1985) [82], have recommended occupational exposure limits which apply to wavelengths between 180 and 400 nm.

The occupational exposure limits of the ACGIH [83] are called threshold limit values (TLVs). All of the exposure limits are for acute effects to the skin and eyes. They are given as a measure of irradiance and radiant exposure. (Irradiance is defined as the level of radiation arriving at a point in space; the quotient of the radiant power and the cross-sectional area; or time-averaged energy flow. Radiant exposure is defined as the change of the incremental radiant energy in a given cross-sectional area; the energy dose of radiant energy.) The units of irradiance are W/m^2; and of radiant exposure, J/m^2.

The ACGIH's TLV for radiant exposure at 254 nm is 60 J/cm^2 or 6.0 mJ/cm^2. (The product of a TLV radiant exposure at a specified wavelength and its relative spectral effectiveness (RSE) is the limiting dose.)

An RSE is a hazard-weighting function indicating the biologic efficacy of that wavelength. The use of RSE's allows irradiance data to be weighted to the limiting TLV at 270 nm of 30 J/m^2, which is based on the potential for photokeratitis.

The NIOSH (USA) [84] recommended exposure limit (REL) requires that the measured irradiance level be multiplied by the RSE at the specific wavelength to obtain the effective irradiance. The maximum permissible exposure time can then be calculated [28]. Neither the REL nor TLV exposure limits should be used to

determine acceptable exposure of photosensitive individuals nor should they be regarded as protective for individuals lacking a crystalline lens.

Qualified personnel should therefore measure irradiance levels from any ultraviolet light source by using a radiometer to determine the exposure levels. Prior to measurement, personnel should determine source characteristics, operator-source interaction and the location of the source relative to reflective objects, such as aluminium surfaces. Should the measurement data warrant use of personal protective equipment for the eyes, qualified personnel can determine which protective eyewear is compatible with the spectral distribution and intensity of the ultraviolet light source.

By appropriate design, however; products utilising UVGI should limit the risk of exposure to UV radiation. Commercially available UV lamps used for germicidal purposes are low-pressure mercury vapour lamps that emit radiant energy in the UV-C range, predominantly at a wavelength of 253.7 nm.

It will be seen that the results plot a straight line on a semi-log graph paper indicating that the equation that describes the phenomenon has the form:

$$N_s/N_o = e^{-Kit} \qquad (A.18)$$

Where:
- N_o = number of bacteria exposed
- N_s = number of bacteria surviving after exposure to UVGI
- i = UV irradiance, $\mu W/cm^2$
- t = time of UV exposure
- K = microbe susceptibility factor, $cm^2/\mu W.s$

This relationship can be used to estimate UVGI effectiveness for inactivating specific airborne infectious micro-organisms when they are exposed to UVGI in mechanically ventilated rooms for various time lengths [57].

The following table provides an abbreviated listing of bacteria ranked in order of their susceptibility to UVGI, i.e. the K values in equation (A.1).

Table A. 2: Values of K (Equation 18) for a selected list of micro-organisms

Micro-organism	**Susceptibility factor $Kcm^2/\mu W.s$**
Bacillus anthracis	0.060
Bacillus subtilis	0.038
Corynebacterium diphtheriae	0.081
Escherichia coli	0.090
Pseudomonas aeruginosa	0.049
Seratia marcescens	0.112
Staphylococcus albus	0.148
Staphylococcus aureus	0.104
Streptococcuc hemolytiscus	0.125

(From Philips lighting. Disinfection by UV-radiation. Eintoven, Holland. Company Bulletin (undated)) [25].

The above equation can be amplified as follows:

$$EAC = -\ln N_s/N_0 = Kit \qquad (A.19)$$

Where; EAC is the number of theoretical effective room air changes that would be required to reduce the number of viable bacteria to the same degree as the UV irradiation alone.

Extrapolating bench experiments carried out at the Harvard School of Public Health [57] have indicated that an optimal upper-room UV installation has the potential of producing the equivalent of 20 or more air ACH in the lower room.

A3.3 Lamps and fixtures for air disinfection with ultraviolet irradiation

Ultraviolet germicidal irradiation (UVGI) may be used in different ways to prevent transmission of airborne communicable diseases.

Lamps having sufficient UV power output may be installed in return-air ducts to disinfect air leaving occupied spaces, especially when the occupants may have unsuspected diseases transmissible by the air route (e.g. hospital out-patient waiting rooms). This application mode may be important when it is necessary to recirculate air from these areas instead of discharging it directly to the atmosphere. However; in-duct air disinfection does relatively little to directly protect occupants in the same room with an infectious source.

Another application is the use of individual fan-cabinet units that recirculate room air over internal UVGI lamps. The use of fan-cabinet room units to effect air disinfection is limited by the number of complete room-air changes they are able to produce, as their fan capacity must be restricted to avoid noise, vibrations and drafts. Under experimental conditions, when these limitations can be ignored, effective clearance can be demonstrated [57].

The most widely promoted application for UVGI is in the form of passive upper-room fixtures containing UVGI lamps that irradiate a horizontal layer of airspace above the occupants and below the ceiling. They are designed to kill or inactivate microbes that enter the upper irradiated zone and are highly dependent on vertical room-air currents to bring susceptible organisms into the irradiated zone.

In rooms lacking mechanical ventilation facilities, there will often be sufficient natural convective mixing to make upper-room UVGI an effective barrier to disease transmission [58]. The lack of modern mechanical ventilation systems was in fact one of the principal attractions for the very early uses of passive upper-room UVGI in hospital wards for patients with contagious diseases [86]. Upper-room applications are considered more favourable over the other modes for the following reason:

- They are passive devices (requiring no fans), easily installed on existing walls and ceilings, readily accessible for inspection and maintenance, silent and inconspicuous, modest in first cost and energy efficient (comparable to fluorescent lighting fixtures). In addition to clinical facilities, they are applicable to all areas where people assemble [87].

- They have advantages over other application modes related to effectiveness in preventing airborne infectious disease transmission within rooms. Most important, infectious microbes are likely to be killed promptly because they are irradiated in the upper region of the room, very close to the locations where they are emitted into the air. Upper-room UVGI makes it unnecessary to depend on airborne microbes becoming air-entrained into fan-cabinet enclosures or return-air ducts containing UVGI lamps. The latter processes depend on the real air mixing rates in contrast to the theoretical air change rate.

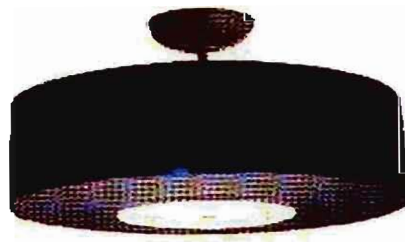

Figure A. 4: A typical ceiling-mounted fixture with 7,5 W or 10 W UV-C lamps to provide 3600 disinfection zone [31]

Upper-room UVGI therefore has the unique potential of killing disease-causing microbes almost as rapidly as they are released to the air and conveyed towards the ceiling. This provides dilution rates of airborne infectious micro-organisms, well beyond the ability of ventilation rates alone, that are still tolerable to occupants [25]. This type of control is comparable to the "direct source control" method described previously, using local exhaust ventilation close to the sources of contaminants to be captured.

Although increasing room-air mixing enhances upper-room UVGI effectiveness, it is clear that the resultant up-flow rate is the most important characteristic, because this is the flow that promptly brings airborne microbes into the lamp's irradiated (killing) zone.

Simple methods for evaluating or predicting the optimum UVGI exposure time required are currently under study [57]. It can be understood that a very rapid passage of lower room air through the upper-room irradiated zone may provide insufficient irradiation time to kill a significant fraction of the entrained infectious organisms, whilst rapid vertical air circulation also implies a rapid return of the same air to the upper irradiated zone of the room for additional exposure.

A rapid upward air movement with a high UV irradiance, that is required to kill all infectious microbes during their first pass through the irradiated zone so they do not re-enter the occupants' breathing zone as viable (and infective) micro-organisms, is the ideal. This is unattainable in practice because the level of UV irradiance is limited due to eye exposure limitations and air mixing is limited due to comfort considerations. Only a fraction of airborne micro-organisms entering the upper irradiated zone will therefore be inactivated during a single pass.

However; even when the destruction of infectious micro-organisms is incomplete during a single pass through the upper irradiated zone, the down-flow of treated air provides dilution of the contagion in the lower, occupied zone as it mixes.

Theoretical modelling indicates that the greatest reduction in infectious micro-organisms in the breathing zone will take place when the greatest rate of air change between the upper and lower room occurs. For rooms that lack adequate air movement, the use of mixing or heater fans may be a satisfactory solution [94].

Fixtures for upper-room installations are designed to accommodate a wide variety of room configurations.

Figure A. 5: A typical wall-mounted fixture [90]

Whilst certain manufacturers are investigating the production of fixtures in South Africa, validated locally manufactured fixtures are as yet not available. The imported UVGI fixtures are equipped with multi-bladed horizontal louvers to confine emissions to a narrow horizontal band and to shield the eyes of occupants from exposure to the bare lamps. These fixtures could feature one or more lamps in a metal enclosure, which also contains the necessary transformer, ballast, switch and wiring.

Many fixtures are equipped with internal, polished mirror-finish aluminium parabolic reflectors to focus lamp emission and increase emission irradiance. Tubular lamps are similar in appearance to standard ceiling fluorescent lighting lamps and come in a variety of lengths and types. The luminous efficiency of these lamps with respect to UVGI is generally about 30% of the lamp rating.

Emission of the UV light is further decreased by blockages caused by the presence of the diffuser. The ratio of luminous flux emitted from the fixtures to that emitted by the lamps is important when comparing different fixtures for disinfection efficacy.

B

Appendix B:
VALIDATION EXPERIMENT: APPARATUS SPECIFICATIONS AND PREPARATION OF BACTERIA CULTURES TO BE AEROSOLISED

Appendix B: VALIDATION EXPERIMENT: APPARATUS SPECIFICATIONS AND PREPARATION OF BACTERIA CULTURES TO BE AEROSOLISED

B1.1 Specifications and description of the apparatus used for the validation experiments

B1.1.1 The MRE-type Collison nebulizer for aerosol generation

A six-yet modified MRE-type Collison nebulizer (BGI Inc., Waltham, Massachusetts) was used to aerosolise the suspensions of Poly-Styrene spheres and bacterial cells. Within the nebulizer, compressed air expands from a pressure of 140 kPa at the stem into six side jets. The reduction of static pressure forces water up the tube at the bottom of the stem, similar to an eductor (an ejector-like device for mixing two fluids, e.g. a liquid and a gas). This liquid suspension is then broken up by the air jet into a dispersion of droplets of very wide size distribution. Most of the droplets are blown onto the internal wall of the glass vessel; however; the minute amount of liquid that escaped compaction comprises only the finest tail of the droplet-size distribution and these droplets are carried up and out of the nebulizer by the spent air.

Because the droplets in the emerging air are aqueous, they evaporate very quickly on admixture with unsaturated air. The wet lifetime of droplets is proportional to diameter squared at any given temperature and relative humidity. The BGI Inc. Collison nebulizer generates droplets of mass median diameter of 2.9μm with a geometric standard deviation of 3.17. Theoretically, predominantly single bacterial cells make their way to the sampler location in the aerosol chamber, a transit time of approximately 6 sec or approximately 10 lifetimes for a 10μm water droplet.

B1.1.2 Uniform Poly-Styrene Latex Micro-spheres (PSL)

The Duke Scientific Corporation PM Standards 4000 Series were sourced as this product is provided with the US National Institute Science and Technology (NIST) traceable particle-size data.

B1.1.3 ARTI HHPC-6 Aerosol Particle Counter

The ARTI® Handheld Particle Counter Six (HHPC-6), simultaneously displays six channels of particle size distribution (0.3, 0.5, 0.7, 1.0, 3.0, 5.0 µm). The HHPC-6 holds 500 samples in data memory and records date, time, counts, sample volume, temperature and Relative humidity. Data is easily downloaded to a computer or printer using a interface cable and utility software. It is often used by among engineering professionals who monitor and verify clean rooms, test filters and track down particle source problems (ART Instruments, Inc., Grants Pass, OR, http://www.arti.net).

B1.1.4 The Anderson 6-STG sampler

Andersen six-stage (6-STGs), viable (microbial) particle sizing samplers, were used to sample the air at specific points along the transfer duct and then in each of the animal exposure chambers during each validation experiment.

The Anderson 6-STG sampler is a multi-orifice, cascade impactor with 400 holes per stage, drawing air at a flow rate of 28.3 L/min. Particle velocity increases as the air flows through successively smaller holes. Large particles (>= 7µm) impact on the first stage and smaller particles follow until accelerated sufficiently to impact at a later stage. This sampler was designed so that all particles collected, regardless of physical size, shape or density, are sized aerodynamically and can be directly related to human lung deposition [88].

B1.1.5 Sampling plates

Sampling plates are prepared by pouring 45ml of Bacto tryptic soy agar (TSA, Difco Laboratories, Michigan, USA) aseptically into each of the six 100mm x 15mm sterile plastic Petri dishes, so that the gap between the nozzles and agar surface meets the manufacturer's specifications. All inside surfaces are maintained in sterile condition until sampling.

B1.2 Preparation of cultures for the Validation Experiments

B1.2.1 *B.subtilis var. niger*

Cultures of *B. subtilis var. niger* were prepared according to the Standard Operating Procedures included in the Experiment's protocol. Briefly, fresh cultures are to be inoculated into 100ml of Bacto trypto soy broth (TSB, Difco Laboratories, Michigan) and incubated for 72hrs at 35°C in Erlenmeyer flasks in a shaker-incubator. The broth is then aseptically transferred to 50ml sterile conical centrifuge tubes (Falcon® 2076, Becton Dickinson, South Africa), capped and centrifuged at 1500g for 10 min. The supernatant is then discarded and the pellet re-suspended in sterile phosphate-buffered water. This washing process is repeated twice more and the cells re-suspended a fourth time in 100ml of sterile phosphate-buffered water. The suspension is then microscopically examined to ensure that it contains motile, single cells with endospores intact and a minute amount of cell debris. The cell concentration is then determined by turbidimetric measurement from previously-developed calibration curves and an appropriate dilution made to attain a total volume of 100ml with a concentration of approximately 106 cells per ml. A dilution series is performed for standard plate count enumeration.[88]

B1.1.2 *Serratia marcescens*

Cultures of *Serratia marcescens* are prepared according to the Standard Operating Procedures included in the Experiment's protocol. Briefly, fresh cultures are inoculated into 100ml of nutrient broth (Difco Laboratories) and incubated for 18-24hrs at 35°C in Erlenmeyer flasks in a shaker-incubator. The broth is then aseptically transferred to 50ml sterile conical centrifuge tubes (Falcon® 2076, Becton Dickinson, South Africa), capped and centrifuged at 1500g for 10 min. The supernatant is then discarded and the pellet resuspended in sterile phosphate-buffered water. This washing process is repeated twice more and the cells resuspended a fourth time in 100ml of sterile phosphate-buffered water. The suspension is then microscopically examined to ensure that it contains motile, single cells and a minute amount of cell debris. The cell concentration is determined by turbidimetric

measurement from previously-developed calibration curves and an appropriate dilution made to attain a total volume of 100ml with a concentration of approximately 106 cells per ml. A dilution series is performed for standard plate count enumeration [89].

C

Appendix C:
CALIBRATION EXPERIMENT:
TUBERCULIN SKIN TEST RESULTS

Appendix C: CALIBRATION EXPERIMENT: TUBERCULIN SKIN TEST RESULTS

Report: 10 Oct 2005, 08:23:43

Each graph was now constructed using those GPs that 'qualify'. In test 3 those animals were used for which qual_3 was equal to 1 (re data set) and in test 4 those animals witjh qual_4 = 1 were used. The graph for test 5 included data for all the animals that were tested again.

TST reader had a intra class correlation of 0.994, based on the original observations, i.e. before rounding to nearest mm.

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. use "E:\Backup_2005\Weyer_Karin_TST\karin_tst_wide_5test_qual.dta", clear

. tab  t2_mean if  t2_mean ~= 0

    t2_mean |      Freq.     Percent        Cum.
------------+-----------------------------------
          5 |          1        0.88        0.88
          6 |          4        3.54        4.42
          7 |          8        7.08       11.50
          8 |         14       12.39       23.89
          9 |         18       15.93       39.82
         10 |         30       26.55       66.37
         11 |         20       17.70       84.07
         12 |         10        8.85       92.92
         13 |          4        3.54       96.46
         14 |          3        2.65       99.12
         15 |          1        0.88      100.00
------------+-----------------------------------
      Total |        113      100.00


. twoway histogram t2_mean if  t2_mean ~= 0, discrete freq
title("Distribution of Reaction Size in Test 2") xtitle("Reaction (mm)
measured as Mean (width & length) in Test 2") xlabel(5(1)15)
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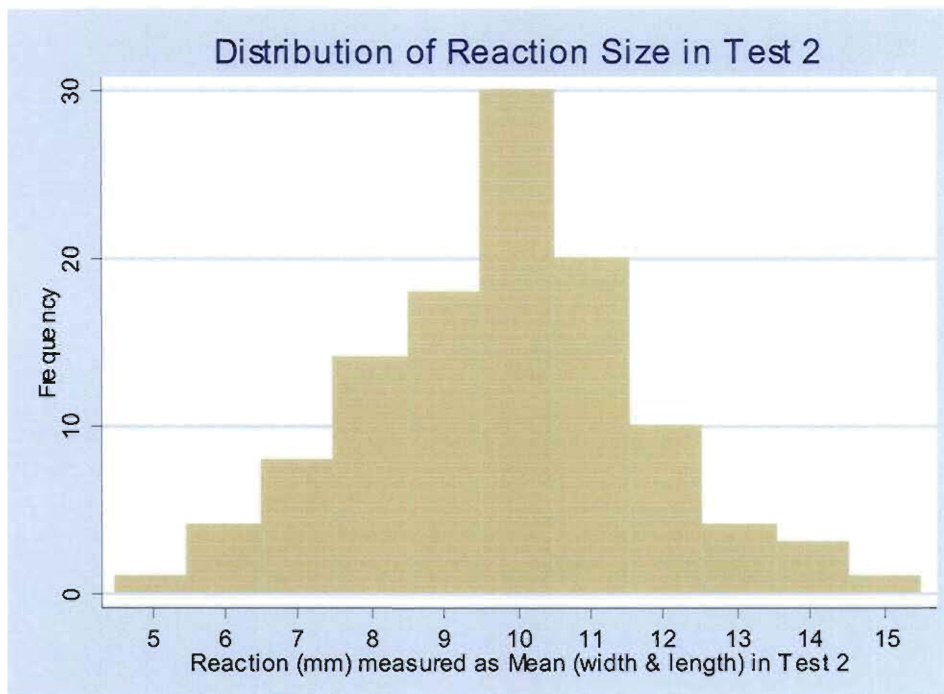
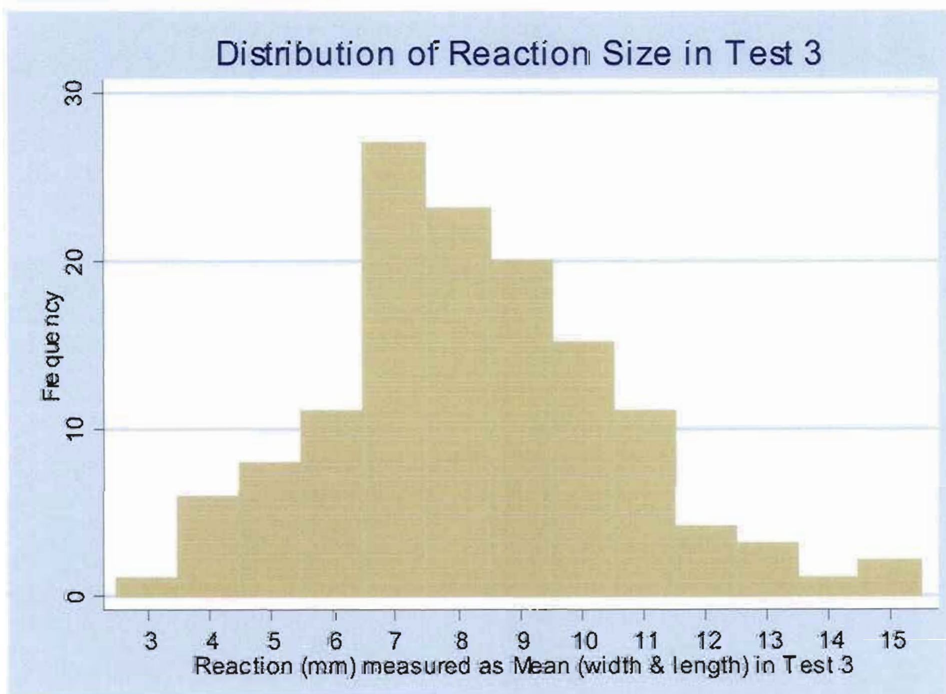


```
. tab  t3_mean if  (t3_mean ~= 0 &  qual_3 == 1)

    t3_mean |      Freq.     Percent        Cum.
------------+-----------------------------------
          3 |          1        0.76        0.76
          4 |          6        4.55        5.30
          5 |          8        6.06       11.36
          6 |         11        8.33       19.70
          7 |         27       20.45       40.15
          8 |         23       17.42       57.58
          9 |         20       15.15       72.73
         10 |         15       11.36       84.09
         11 |         11        8.33       92.42
         12 |          4        3.03       95.45
         13 |          3        2.27       97.73
         14 |          1        0.76       98.48
         15 |          2        1.52      100.00
------------+-----------------------------------
      Total |        132      100.00
```

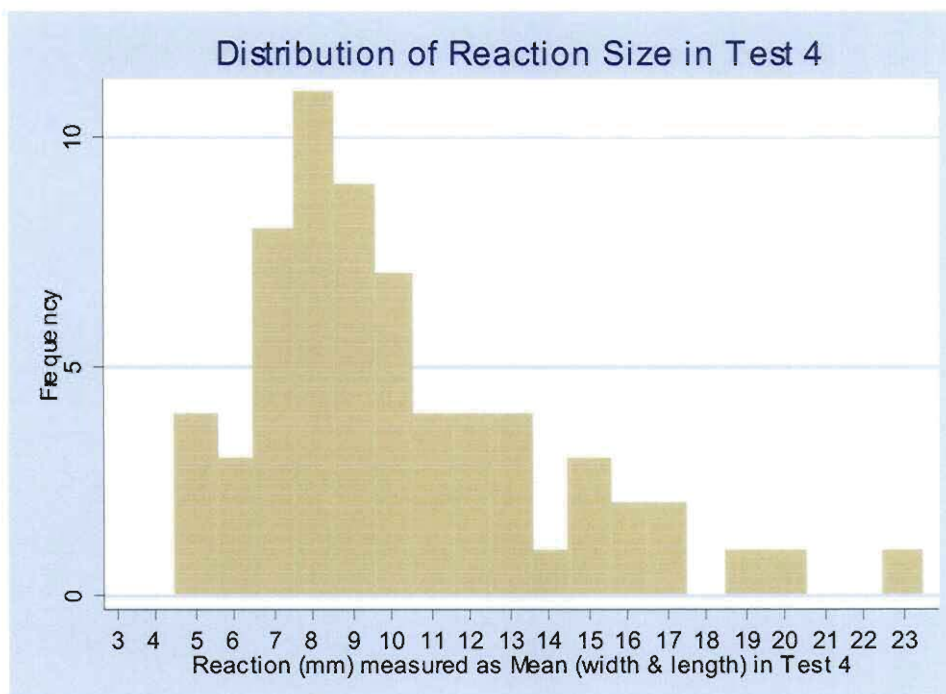
```
. twoway histogram t3_mean if  (t3_mean ~= 0 &  qual_3 == 1), discrete freq
title("Distribution of Reaction Size in Test 3") xtitle("Reaction (mm)
measured as Mean (width & length) in Test 3") xlabel(3(1)15)
```



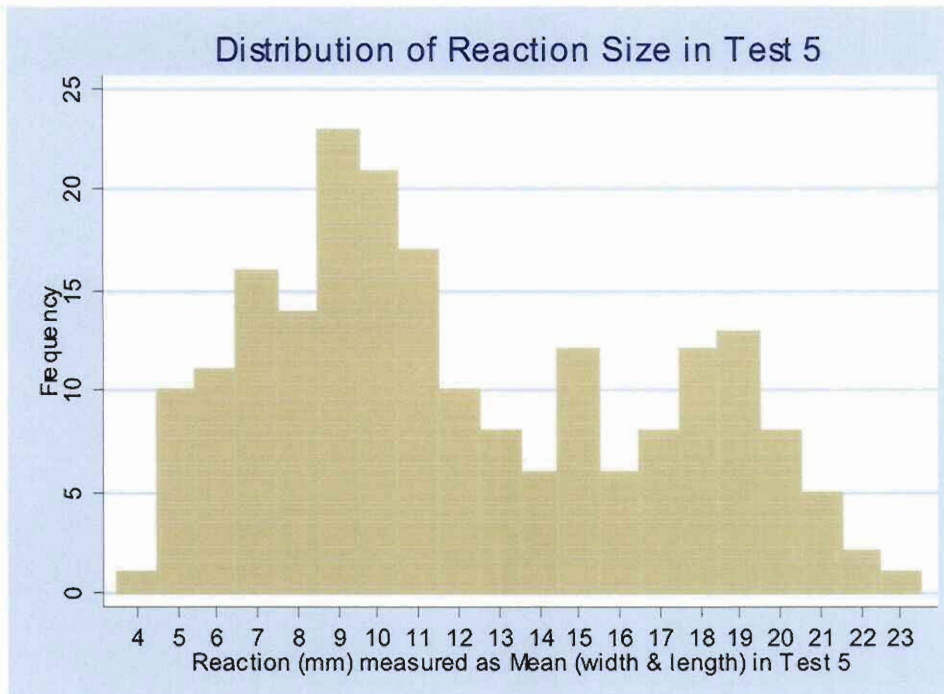
```
. tab  t4_mean if  (t4_mean ~= 0 &  qual_4 == 1)

    t4_mean |      Freq.     Percent        Cum.
------------+-----------------------------------
          5 |          4        6.15        6.15
          6 |          3        4.62       10.77
          7 |          8       12.31       23.08
          8 |         11       16.92       40.00
          9 |          9       13.85       53.85
         10 |          7       10.77       64.62
         11 |          4        6.15       70.77
         12 |          4        6.15       76.92
         13 |          4        6.15       83.08
         14 |          1        1.54       84.62
         15 |          3        4.62       89.23
         16 |          2        3.08       92.31
         17 |          2        3.08       95.38
         19 |          1        1.54       96.92
         20 |          1        1.54       98.46
         23 |          1        1.54      100.00
------------+-----------------------------------
      Total |         65      100.00
```

```
. twoway histogram t4_mean if  (t4_mean ~= 0 &  qual_4 == 1), discrete freq
title("Distribution of Reaction Size in Test 4") xtitle("Reaction (mm)
measured as Mean (width & length) in Test 4") xlabel(3(1)15)
```



```
. twoway histogram t5_mean if  (t5_mean ~= 0), discrete freq
title("Distribution of Reaction Size in T
> est 5") xtitle("Reaction (mm) measured as Mean (width & length) in Test
5") xlabel(4(1)23)
```



. list

	id	t2_mean	qual_3	t3_mean	qual_4	t4_mean	t5_mean
1.	1	11	0	.	0	.	21
2.	2	0	1	0	1	8	.
3.	3	10	0	11	0	.	15
4.	4	10	0	.	0	.	10
5.	5	10	0	.	0	.	10
6.	6	0	1	0	1	0	0
7.	7	0	1	0	1	.	.
8.	8	0	1	8	1	10	0
9.	9	0	1	6	1	5	0
10.	10	9	1	8	1	0	9
11.	11	0	1	0	1	0	0
12.	12	7	1	0	1	0	8
13.	13	0	1	0	1	.	19
14.	14	0	1	7	1	.	21
15.	15	0	1	6	1	0	.
16.	16	0	1	.	1	.	5
17.	17	0	1	0	1	0	0
18.	18	11	0	11	0	13	16
19.	19	0	1	9	1	11	9
20.	20	0	1	4	1	0	15
21.	21	0	1	8	1	8	10
22.	22	9	1	0	1	0	12
23.	23	0	1	8	1	0	10
24.	24	10	0	.	0	.	7
25.	25	0	1	0	1	0	15
26.	26	0	1	0	1	11	18
27.	27	0	1	0	1	9	10
28.	28	8	1	9	1	9	6
29.	29	11	0	.	0	.	.
30.	30	0	1	7	1	.	7
31.	31	0	1	0	1	0	0
32.	32	0	1	0	1	10	0
33.	33	6	1	8	1	10	0
34.	34	6	1	0	1	0	0
35.	35	7	1	7	1	9	8
36.	36	0	1	0	1	0	0
37.	37	0	1	9	1	0	7
38.	38	0	1	9	1	8	11
39.	39	0	1	3	1	23	20
40.	40	0	1	0	1	7	0
41.	41	0	1	0	1	0	0
42.	42	0	1	0	1	5	0
43.	43	10	0	.	0	.	14
44.	44	0	1	0	1	0	0
45.	45	10	0	.	0	.	14
46.	46	0	1	9	1	7	22
47.	47	0	1	0	1	0	.
48.	48	10	0	.	0	.	.
49.	49	0	1	0	1	0	0

50.	50	9	1	10	0	.	0
51.	51	0	1	9	1	0	0
52.	52	0	1	7	1	9	10
53.	53	0	1	.	1	15	17
54.	54	0	1	0	1	12	13
55.	55	0	1	8	1	13	18
56.	56	0	1	0	1	0	7
57.	57	0	1	0	1	0	5
58.	58	0	1	0	1	0	0
59.	59	0	1	8	1	19	18
60.	60	9	1	12	0	.	6
61.	61	0	1	12	0	.	10
62.	62	0	1	6	1	0	0
63.	63	0	1	5	1	0	0
64.	64	0	1	0	1	0	0
65.	65	0	1	0	1	0	8
66.	66	0	1	9	1	9	16
67.	67	9	1	10	0	.	19
68.	68	8	1	10	0	10	6
69.	69	0	1	0	1	0	11
70.	70	10	0	.	0	.	8
71.	71	0	1	9	1	20	15
72.	72	0	1	0	1	0	18
73.	73	0	1	0	1	0	0
74.	74	8	1	9	1	7	0
75.	75	7	1	0	1	0	0
76.	76	10	0	.	0	0	.
77.	77	0	1	0	1	0	0
78.	78	0	1	0	1	0	8
79.	79	0	1	0	1	0	0
80.	80	0	1	0	1	0	5
81.	81	10	0	.	0	.	21
82.	82	11	0	.	0	.	18
83.	83	0	1	0	1	9	18
84.	84	0	1	0	1	0	9
85.	85	10	0	9	0	0	0
86.	86	13	0	.	0	.	20
87.	87	0	1	0	1	0	8
88.	88	0	1	0	1	0	9
89.	89	0	1	10	0	.	9
90.	90	12	0	.	0	.	.
91.	91	0	1	8	1	0	10
92.	92	0	1	0	1	0	0
93.	93	0	1	0	1	0	0
94.	94	0	1	0	1	6	7
95.	95	11	0	.	0	.	11
96.	96	0	1	0	1	0	0
97.	97	9	1	9	1	0	0
98.	98	0	1	0	1	15	15
99.	99	0	1	9	1	7	0
100.	100	0	1	11	0	.	0
101.	101	0	1	0	1	0	7
102.	102	0	1	0	1	0	0

103.	103	9	1	10	0	9	15
104.	104	0	1	8	1	16	17
105.	105	0	1	6	1	8	7
106.	106	9	1	11	0	.	12
107.	107	0	1	0	1	0	0
108.	108	0	1	0	1	0	0
109.	109	0	1	0	1	0	.
110.	110	0	1	0	1	0	11
111.	111	0	1	0	1	6	8
112.	112	0	1	0	1	0	5
113.	113	0	1	0	1	0	6
114.	114	0	1	0	1	0	0
115.	115	10	0	0	0	11	11
116.	116	8	1	9	1	15	17
117.	117	0	1	0	1	0	0
118.	118	11	0	.	0	.	15
119.	119	9	1	.	1	.	20
120.	120	0	1	0	1	0	0
121.	121	8	1	11	0	.	15
122.	122	10	0	.	0	.	10
123.	123	0	1	0	1	0	18
124.	124	0	1	7	1	12	14
125.	125	0	1	7	1	0	9
126.	126	0	1	7	1	0	9
127.	127	.	1	10	0	11	14
128.	128	0	1	10	0	8	18
129.	129	0	1	0	1	0	0
130.	130	0	1	0	1	11	5
131.	131	7	1	13	0	9	9
132.	132	8	1	15	0	8	8
133.	133	0	1	6	1	0	0
134.	134	0	1	7	1	0	12
135.	135	0	1	7	1	8	13
136.	136	5	1	0	1	0	0
137.	137	0	1	0	1	8	0
138.	138	0	1	0	1	0	0
139.	139	0	1	0	1	0	0
140.	140	0	1	10	0	.	8
141.	141	0	1	0	1	0	0
142.	142	0	1	11	0	.	8
143.	143	0	1	8	1	0	11
144.	144	0	1	0	1	0	5
145.	145	11	0	.	0	.	13
146.	146	8	1	7	1	0	6
147.	147	10	0	.	0	.	19
148.	148	0	1	.	1	0	11
149.	149	9	1	.	1	.	.
150.	150	0	1	.	1	6	0
151.	151	0	1	10	0	0	11
152.	152	0	1	7	1	0	5
153.	153	11	0	.	0	.	.
154.	154	0	1	0	1	0	0
155.	155	0	1	0	1	0	7

156.	156	0	1	0	1	0	19
157.	157	0	1	0	1	0	0
158.	158	7	1	0	1	8	0
159.	159	0	1	0	1	7	0
160.	160	0	1	7	1	0	0
161.	161	0	1	7	1	0	14
162.	162	0	1	0	1	0	0
163.	163	0	1	8	1	0	9
164.	164	0	1	0	1	7	9
165.	165	0	1	0	1	0	0
166.	166	0	1	10	0	.	10
167.	167	0	1	0	1	0	13
168.	168	0	1	0	1	13	12
169.	169	10	0	.	0	.	9
170.	170	0	1	6	1	0	0
171.	171	0	1	0	1	0	9
172.	172	9	1	.	1	.	0
173.	173	13	0	.	0	.	0
174.	174	0	1	0	1	0	0
175.	175	0	1	.	1	0	0
176.	176	0	1	.	1	8	10
177.	177	0	1	.	1	0	0
178.	178	0	1	0	1	0	0
179.	179	9	1	.	1	.	.
180.	180	0	1	13	0	.	.
181.	181	0	1	0	1	0	0
182.	182	0	1	0	1	0	0
183.	183	0	1	6	1	10	0
184.	184	0	1	0	1	0	20
185.	185	0	1	0	1	5	0
186.	186	0	1	0	1	7	0
187.	187	0	1	9	1	.	9
188.	188	9	1	10	0	.	15
189.	189	0	1	0	1	0	0
190.	190	0	1	5	1	0	0
191.	191	11	0	.	0	.	9
192.	192	0	1	0	1	0	0
193.	193	0	1	4	1	0	4
194.	194	0	1	0	1	0	7
195.	195	8	1	6	1	0	0
196.	196	10	0	.	0	.	.
197.	197	7	1	0	1	0	0
198.	198	10	0	.	0	.	0
199.	199	0	1	7	1	0	0
200.	200	0	1	10	0	.	20
201.	201	0	1	0	1	0	0
202.	202	0	1	4	1	0	6
203.	203	0	1	0	1	9	7
204.	204	6	1	0	1	0	0
205.	205	0	1	5	1	0	0
206.	206	11	0	.	0	.	15
207.	207	0	1	5	1	0	17
208.	208	11	0	.	0	.	8
209.	209	10	0	0	0	9	0

210.	210	0	1	8	1	10	13
211.	211	7	1	11	0	.	12
212.	212	0	1	.	1	0	19
213.	213	0	1	.	1	0	7
214.	214	0	1	7	1	0	7
215.	215	0	1	5	1	0	9
216.	216	0	1	13	0	.	21
217.	217	0	1	8	1	0	5
218.	218	0	1	12	0	.	11
219.	219	0	1	0	1	0	0
220.	220	0	1	0	1	0	0
221.	221	7	1	10	0	.	19
222.	222	8	1	8	1	11	10
223.	223	0	1	0	1	0	6
224.	224	0	1	6	1	0	0
225.	225	0	1	0	1	0	18
226.	226	0	1	9	1	14	23
227.	227	0	1	0	1	0	0
228.	228	0	1	0	1	0	0
229.	229	10	0	.	0	.	10
230.	230	0	1	0	1	0	0
231.	231	11	0	.	0	.	10
232.	232	0	1	11	0	.	0
233.	233	0	1	4	1	0	0
234.	234	0	1	5	1	9	10
235.	235	0	1	5	1	0	11
236.	236	0	1	0	1	0	16
237.	237	0	1	0	1	0	0
238.	238	0	1	0	1	0	0
239.	239	0	1	5	1	.	0
240.	240	11	0	.	0	.	.
241.	241	0	1	0	1	0	0
242.	242	11	0	.	0	.	16
243.	243	8	1	9	1	.	9
244.	244	0	1	8	1	0	0
245.	245	11	0	.	0	.	12
246.	246	12	0	.	0	.	12
247.	247	0	1	0	1	0	0
248.	248	0	1	0	1	0	0
249.	249	0	1	0	1	0	11
250.	250	12	0	.	0	.	.
251.	251	10	0	0	0	0	9
252.	252	0	1	8	1	0	10
253.	253	0	1	15	0	.	.
254.	254	0	1	7	1	0	10
255.	255	0	1	0	1	0	0
256.	256	0	1	0	1	7	9
257.	257	0	1	0	1	0	11
258.	258	0	1	7	1	0	0
259.	259	9	1	8	1	0	0
260.	260	0	1	0	1	0	0
261.	261	0	1	7	1	0	0
262.	262	0	1	0	1	0	16

263.	263	0	1	0	1	0	7
264.	264	0	1	0	1	0	0
265.	265	0	1	12	0	.	18
266.	266	0	1	7	1	0	19
267.	267	0	1	7	1	0	0
268.	268	0	1	0	1	0	0
269.	269	0	1	0	1	0	13
270.	270	0	1	0	1	0	0
271.	271	0	1	14	0	.	20
272.	272	10	0	.	0	.	9
273.	273	0	1	7	1	.	11
274.	274	12	0	.	0	.	.
275.	275	15	0	.	0	.	19
276.	276	.	0	.	0	0	0
277.	277	0	1	.	1	.	0
278.	278	10	0	.	0	14	17
279.	279	13	0	.	0	.	8
280.	280	0	1	0	1	0	0
281.	281	0	1	9	1	0	0
282.	282	0	1	0	1	0	0
283.	283	11	0	13	0	.	18
284.	284	0	1	8	1	0	0
285.	285	14	0	.	0	.	.
286.	286	0	1	0	1	0	0
287.	287	0	1	0	1	0	0
288.	288	10	0	.	0	.	11
289.	289	0	1	0	1	0	0
290.	290	0	1	0	1	0	0
291.	291	0	1	0	1	0	0
292.	292	0	1	7	1	17	20
293.	293	8	1	4	1	0	0
294.	294	10	0	8	0	0	6
295.	295	14	0	.	0	.	.
296.	296	0	1	10	0	.	9
297.	297	0	1	4	1	0	8
298.	298	8	1	9	1	10	0
299.	299	0	1	8	1	0	0
300.	300	10	0	10	0	.	0
301.	301	11	0	0	0	.	13
302.	302	0	1	0	1	0	0
303.	303	0	1	0	1	0	0
304.	304	0	1	11	0	0	8
305.	305	0	1	9	1	10	0
306.	306	8	1	0	1	8	0
307.	307	0	1	8	1	9	0
308.	308	9	1	0	1	0	7
309.	309	0	1	6	1	13	17
310.	310	0	1	7	1	0	0
311.	311	10	0	.	0	.	9
312.	312	0	1	0	1	0	0
313.	313	8	1	.	1	12	15
314.	314	10	0	.	0	.	11
315.	315	12	0	.	0	.	12

316.	316	9	1	.	1	.	.
317.	317	0	1	0	1	5	10
318.	318	12	0	.	0	.	.
319.	319	0	1	9	1	8	10
320.	320	0	1	0	1	17	22
321.	321	0	1	.	1	.	0
322.	322	10	0	.	0	.	13
323.	323	10	0	.	0	.	17
324.	324	0	1	0	1	0	0
325.	325	12	0	.	0	.	12
326.	326	11	0	.	0	.	19
327.	327	0	1	8	1	0	11
328.	328	0	1	6	1	0	0
329.	329	0	1	8	1	0	0
330.	330	0	1	7	1	0	0
331.	331	0	1	7	1	0	6
332.	332	0	1	0	1	0	7
333.	333	0	1	0	1	0	0
334.	334	13	0	.	0	.	15
335.	335	0	1	0	1	0	0
336.	336	0	1	0	1	0	0
337.	337	0	1	0	1	0	7
338.	338	9	1	9	1	0	17
339.	339	0	1	0	1	0	0
340.	340	0	1	0	1	0	6
341.	341	0	1	10	0	.	11
342.	342	9	1	.	1	.	5
343.	343	0	1	8	1	13	18
344.	344	12	0	.	0	.	12
345.	345	0	1	11	0	.	19
346.	346	14	0	.	0	.	19
347.	347	0	1	0	1	0	6
348.	348	0	1	11	0	.	10
349.	349	0	1	0	1	0	0
350.	350	11	0	.	0	0	10
351.	351	6	1	11	0	.	9
352.	352	10	0	.	0	.	20
353.	353	0	1	7	1	16	19
354.	354	0	1	7	1	8	5
355.	355	0	1	11	0	.	21
356.	356	12	0	.	0	.	16
357.	357	0	1	0	1	12	0
358.	358	11	0	12	0	.	14
359.	359	0	1	0	1	.	19
360.	360	12	0	9	0	9	9

Intraclass correlation for Kobus = 0.994

```
. use "E:\Backup_2005\Weyer_Karin_TST\test5_intra.dta", clear

. loneway  t5mean id if  t5mean ~= 0

                   One-way Analysis of Variance for t5mean:

                                                Number of obs =       408
                                                    R-squared =    0.9969

    Source                 SS          df       MS            F      Prob > F
-------------------------------------------------------------------------
Between id             9327.4273     203    45.947918     320.62      0.0000
Within id              29.235036     204     .143309
-------------------------------------------------------------------------
Total                  9356.6624     407    22.989342

         Intraclass       Asy.
         correlation      S.E.        [95% Conf. Interval]
         ------------------------------------------------
            0.99378      0.00087       0.99208      0.99548


. xtreg  t5mean if   t5mean ~= 0 , i( id ) mle

Random-effects ML regression                      Number of obs        =
408
Group variable (i): id                            Number of groups     =
204

Random effects u_i ~ Gaussian                     Obs per group: min =
2
                                                                 avg =
2.0
                                                                 max =
2

                                                  Wald chi2(0)         =
0.00
Log likelihood  = -770.67084                      Prob > chi2          =
.

---------------------------------------------------------------------------
--
      t5mean |      Coef.   Std. Err.      z    P>|z|      [95% Conf.
Interval]
-------------+-------------------------------------------------------------
--
       _cons |   12.11045   .3347617    36.18   0.000     11.45433
12.76657
-------------+-------------------------------------------------------------
--
    /sigma_u |   4.773855   .2370853    20.14   0.000     4.309176
5.238533
    /sigma_e |   .3785618   .0187415    20.20   0.000     .3418291
.4152944
-------------+-------------------------------------------------------------
--
```

```
         rho |    .993751   .0008723                      .9918196
.9952671
------------------------------------------------------------------------
Likelihood-ratio test of sigma_u=0: chibar2(01)=  894.60
Prob>=chibar2 = 0.000
```

D

Appendix D:
INVESTIGATING WELLS/RILEY EQUATION TO DETERMINE THE SPREAD OF AIRBORNE INFECTION USING THE RESULTS FROM THE CALIBRATION EXPERIMENT

Appendix D: INVESTIGATING WELLS/RILEY EQUATION TO DETERMINE THE SPREAD OF AIRBORNE INFECTION USING THE RESULTS FROM THE CALIBRATION EXPERIMENT

D.1 Introduction

The aim of this part of the study was to investigate the use of the Wells/Riley equation, as discussed in Chapter 2 of this thesis, in determining the spread of airborne infection. The work focused specifically on whether the equation describes the situation recorded during the calibration experiment at the AIR facility.

Appendix D deals with the "simulation" work done to understand the patterns described by the Wells/Riley equation, as well as the more formal statistical approach of analyzing the data with survival analysis techniques.

D.2 Investigating the Wells/Riley equation

The original equation proposed to model the number of infections obtained from airborne transmission of a disease [50] is as follows:

$$\frac{C}{S} = 1 - e^{(-Ipqt/Q)} \qquad (2.9)$$

Where:

C	= number infected
S	= number of susceptibles
I	= number of cases in the infectious stage (number of infectors)
t	= exposure time
q	= number of doses of airborne infection (quanta) or rate of production of infectious doses
Q	= removal rate by fresh air ventilation (volume per unit time)
p	= pulmonary ventilation per susceptible (volume per unit time)
r	= rate of infection per infector or effective contact rate

This equation was simplified by setting $r = \frac{pq}{Q}$ to be:

$$\frac{C}{S} = 1 - e^{(-Irt)} \tag{2.10}$$

Where:

C	= number infected
S	= number of susceptibles
I	= number of cases in the infectious stage (number of infectors)
t	= exposure time
r	= rate of infection per infector or effective contact rate

D.2.1 Using a small group to simulate the Wells/Riley equation over a 33 week period

In order to investigate the use of the Wells/Riley equation, and to establish whether it seems to model a practical situation well, a small "simulation" was run. A group of 50 susceptibles and 6 infectors were used, assuming none of the susceptibles were infected at the start of the period and assuming a value for r. The pattern of infection over time (i.e. differing values of t) was derived using the Wells/Riley equation. An example is shown in Table D.1 for a period of 4 weeks.

Table D. 1: Simulated pattern of infection over time (4 weeks)

Using r = 0.01	**Week number**				
	0	**1**	**2**	**3**	**4**
Number newly infected in week (C)	0	3	5	7	7
Number of remaining susceptibles (S)	50	50	47	42	35
Cumulative value for C	0	3	8	15	22

The table shows the following:

- Week 0 signifies the start of the time period. There are 50 susceptibles (*S*) and 0 infections (*C*).

- Week 1 starts with 50 susceptibles. Applying Riley's formula with S = 50, I = 6, r = 0.01 and t = 1, a value of 3 is derived for the number of infections (*C*). Those 3 infections are removed from the susceptible group, leaving 47 susceptibles at the end of week 1, or at the start of week 2.

- The value of ***S*** therefore changes for week 2, and ***t*** changes to ***t*** = 2 in week 2, but ***I*** and ***r*** remain the same. This gives ***C*** = 5 in week 2 from Riley's equation. The cumulative value for ***C*** (total number of infections recorded since week 0) in week 2 is the 3 infections of week 1 plus the 5 infections of week 2, or a total of 8 infections.

- In week 3, the number of susceptibles change to 42 and ***t*** changes to ***t*** = 3, while ***I*** and ***r*** remain the same. The number of new infections is now 7 and the cumulative number of infections becomes 3 + 5 + 7 = 15.

The process can be repeated until the whole group of susceptibles are infected or until a certain number of weeks have passed, or a certain number of infections have been recorded.

The above example shows that even though the number of susceptibles is lower each week, the number of infections ***C*** continually grows. Since ***I*** and ***r*** remain the same, the increase in number of infections is mainly due to the length of time of exposure to infection ***t***, which can be expected. However; if as time goes by the length of exposure increases but the number of susceptibles become smaller, the infections will drop off over time. It therefore seems that the equation describes the process of infection quite well. The Figure D.1 shows the pattern of the cumulative number of infections from "simulation runs" lasting 33 weeks with different values of ***r***. The number of susceptibles at the start of the period was set to 50.

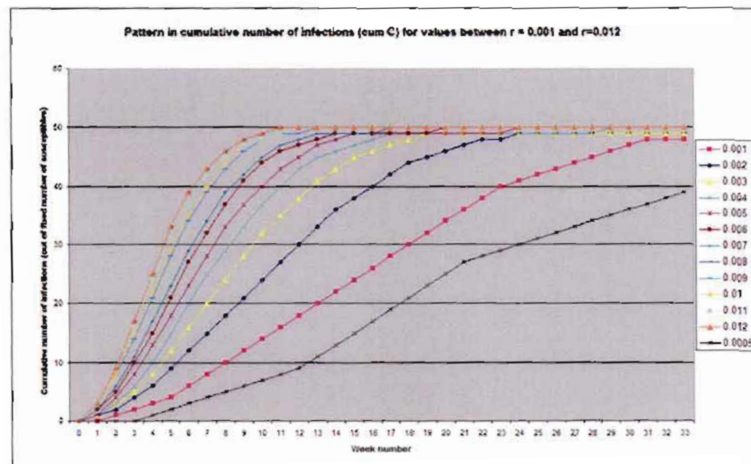


Figure D. 1: Pattern in cumulative number of infections (cum C) for values of ***r*** *between 0.001 and 0.012*

The graph shows that the cumulative infection increases quite sharply in early weeks for high values of ***r***, while less sharply for lower values of ***r***. It takes roughly 12 weeks for the whole group of susceptibles to be infected if ***r*** = 0.012, while almost 30 weeks if ***r*** = 0.001.

D.2.2 Using 360 guinea pigs, (i.e. the same number used during the calibration experiment), to simulate the Wells/Riley equation over a 33 week period

The Figure D.2 indicates similar patterns of cumulative infections from repeating "simulation runs" for different ***r*** values, but this time using a starting group of 360 susceptibles.

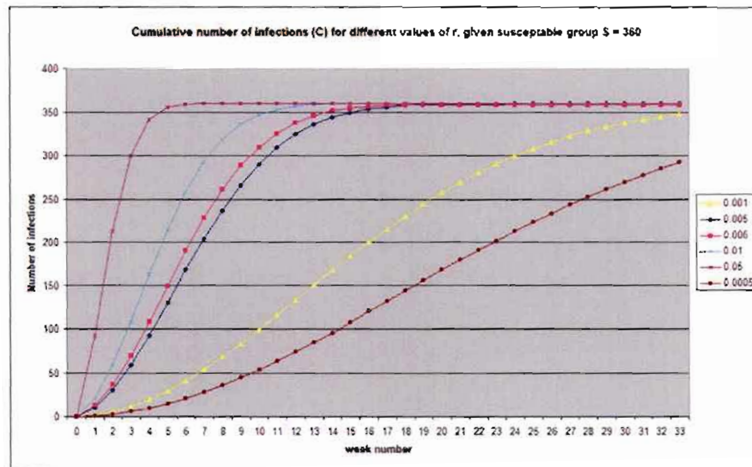


Figure D. 2: Cumulative number of infections C for different values of ***r****, for the given susceptible group S = 360*

D.2.3 Using 360 guinea pigs, (i.e. the same number used during the calibration experiment), to simulate the Wells/Riley equation over a 14 week period

In order to link up the information obtained from these simulations with the information recorded during the calibration experiment of the AIR facility, the portion of the Figure D.2 that would be visible after conducting the experiment for 14 weeks was extracted from the Figure D.2 above, and can be seen below.

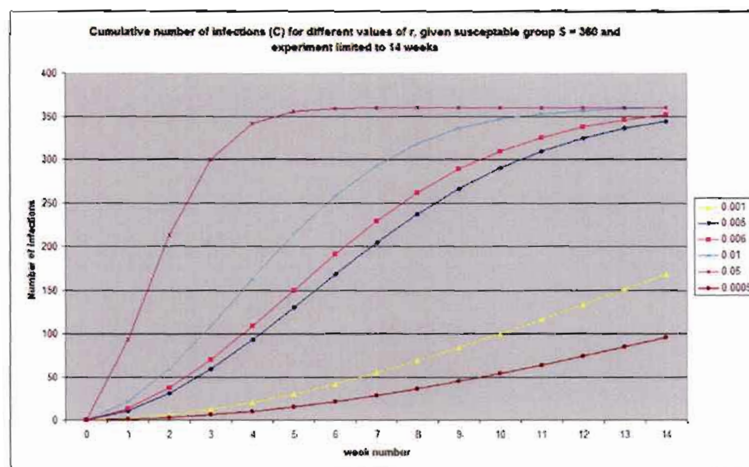


Figure D. 3: Cumulative number of infections (C) for different values of ***r****, for the given susceptible group S = 360 and experiment limited to 14 weeks*

D.2.4 Using the observed data from the calibration experiment to simulate the Wells/Riley equation, using 360 guinea pigs over the14 week period

Since the number of susceptibles in the calibration test was 360 and was carried out over 14 weeks, the pattern obtained from the data can be compared to the "simulation runs" for 360 susceptibles over 14 weeks. It seems as if a roughly similar pattern was followed by the actual data as was seen for the lower ***r*** values on the simulated data graph Figure D.4. Specifically, it appears as if an r value of between 0.001 and 0.0005 may describe the actual data measured.

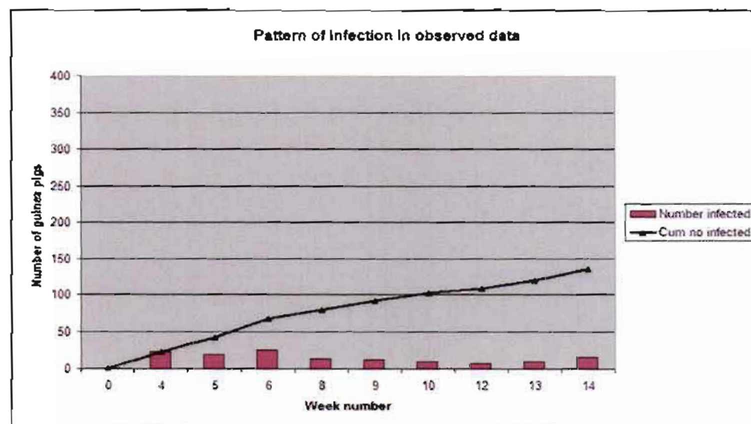


Figure D. 4: Pattern of infection in observed data

To investigate this further, the value of ***r*** was calculated for the actual data for each week, using the Wells/Riley equation and taking into account the number of infections and number of susceptibles in each week. Figure D.5 indicates these calculated values of ***r*** for each week, as recorded in the actual AIR calibration data.

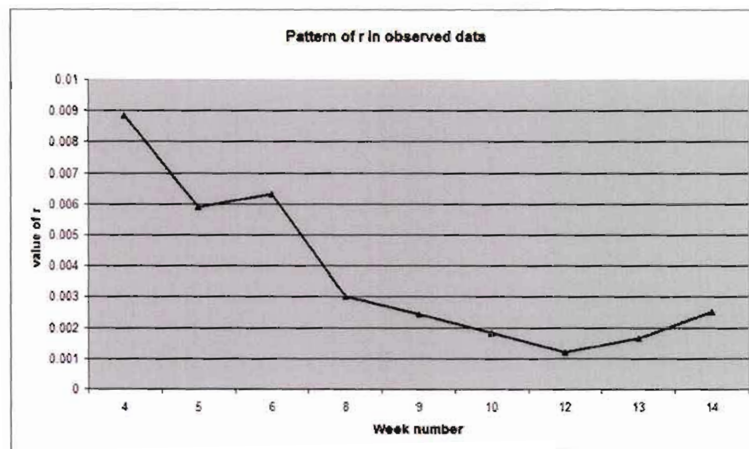


Figure D. 5: Pattern of ***r*** *in the observed data*

The graph seems to indicate that the value of r is not constant over the whole recorded period, taking on values of between 0.001 and 0.009 over the period.

The information displayed in Figure D.5 is important, since the Wells/Riley equation would require a constant value for ***r*** over the whole experimental period, and the actual data seems to indicate that ***r*** was not constant. This aspect was further investigated by means of a survival analysis on the data, as described in the next subsection.

D.3 Survival analysis

D.3.1 Data manipulation

Due to the fact that the guinea pigs used in the calibration experiment were tested at specific intervals, the exact time at which a guinea pig became infected is not known. As a result the data recorded in the calibration test was not continuous-time data and needed to be grouped into intervals. The data on infected guinea pigs for the three tests were grouped into time intervals and summarized into the format given in the Table D.2 below.

All guinea pigs were known to be uninfected at time 0 but although the guinea pigs were tested, for example, at weeks 4, 5 and 6, only roughly one third were tested on

each of these weeks and thus the results of the entire set of guinea pigs were only known after week 6. Hence all the results from weeks 4, 5 and 6 are grouped into the time interval 0-6. The results were similarly grouped for the other tests. Guinea pigs left uninfected at the end of the study are classified as censored.

Table D. 2: Actual data from the calibration experiment

No. of weeks (interval)	Number of infections	Status (0=infected	
1=censored)	Interval midpoint		
0 – 6	67	0	3
6 – 10	35	0	8
10 – 14	33	0	12
10 – 14	222	1	12

One of the problems with this dataset is that you are left with only three time intervals which is not sufficient information from which to derive reliable results. The fact that the nature of the study forces one to use grouped data also restricts the type of survival analysis techniques that can be used. One of the techniques that can be fitted to grouped data is a life table and for this a midpoint value was also assigned to each interval, as shown in the table.

D.3.2 Results

Using the grouped or interval data that was created from the calibration test data, a life table was fitted. Although the sample is too small to do a full statistical analysis, one can illustrate the use of this technique for future analysis.

The results obtained from this technique produced the following Table D.3:

Table D. 3: Conditional probability of being infected

Life Table Survival Estimates								
Interval		No. Failed	No. Censored	Effective Sample Size	Conditional Probability of Failure	Conditional Probability Standard Error	Evaluated at the Midpoint of the Interval	
Lower	Upper						Hazard	Hazard Standard Error
0	**6**	67	0	357	0.1877	0.0207	0.034518	0.004194
6	**10**	35	0	290	0.1207	0.0191	0.03211	0.005416
10	**14**	33	222	144	0.2292	0.0350	0.064706	0.011169

Table D.3 gives the conditional probability of failure (in this case probability of being infected) as well as the hazard function for each interval of time. The hazard function can be defined as the risk of infection at time t and from the table one can see that the hazard is not constant over time but increases in the third time interval. This is also displayed in Figure D.6 of the hazard function shown below.

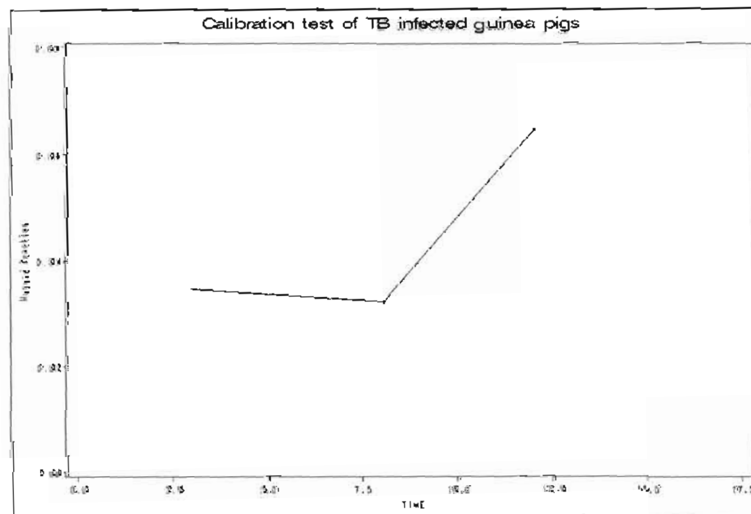


Figure D. 6: Calibration test of TB infected guinea pigs

D.4 Conclusions

Although the hazard changes over time, the increase in the hazard or risk of infection in the last observation may be inflated due to the termination of the study after the third test. As a result, there is a need for more time studies to be conducted on the guinea pigs to get a better idea of how the hazard function changes over time.

One of the reasons why the hazard function is of interest is because the Wells/Riley equation uses an exponential survival distribution that implies that the hazard does not change over time. If a more detailed study can reveal that the hazard is in fact not constant over time then one might have a basis for contradicting the Wells/Riley equation. One would then be able to fit a more appropriate survival distribution to the TB data which would include an additional parameter that can describe the change in hazard or risk of infection over time.

D.5 Recommendations

By conducting a longer study with more frequent tests using a set of guinea pigs and where each guinea pig is tested for infection at all time intervals, until infection takes place or the study terminates, an approximation of continuous-time data could be obtained. With such data one could fit various survival distributions to the data in order to determine whether there is a more appropriate distribution for the risk of TB infection than that presented by the exponential distribution. From such a study a better estimate of the hazard function could be obtained and in order to test whether it remains constant over time.

It needs to be emphasized, as motivation for the data collection as described above, that one of the conditions for the use of these types of survival analysis techniques is that each uninfected guinea pig is retested in each successive time interval until the guinea pig becomes infected or until the end of the study. If such an implementation of guinea pig testing is not appropriate then other analysis techniques would need to be investigated.